UNIVERSITY OF ALBERTA

THE PHARMACOKINETICS OF 2-ARYLPROPIONIC ACID ENANTIOMERS: FLURBIPROFEN AND FENOPROFEN

BY

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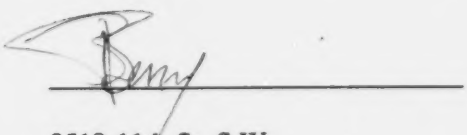

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'The Heavens declare the Glory of God;

and the firmament shows His handiwork'

Psalm 19:1 (NKJV)

'life cannot and never could exist

without molecular dissymmetry'

Terent'ev and Labunovskii 1957

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled THE PHARMACOKINETICS OF 2-ARYLPROPIONIC ACID ENANTIOMERS: FLURBIPROFEN AND FENOPROFEN submitted by BRIAN WILLIAM BERRY in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in PHARMACEUTICAL SCIENCES (PHARMACOKINETICS).

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Dedication

To my children, Megan and David, whose innocence, sense of wonder, curiosity and insatiable desire to learn, continually provided me with strength for the journey.

To my brother Michael, whom along with the support and encouragement of his family, demonstrated a heroic spirit and an unmoving tenacity for life, and in memory will continue to be a personal source of inspiration.

Abstract

Generally, a relationship between therapeutic response and plasma concentrations of non-steroidal anti-inflammatory drugs (NSAIDs) has been difficult to achieve with wide ranging explanations. Particularly with the 2-arylpropionic acid (2-APA) class of NSAIDs, possession of a chiral carbon and general clinical administration as a 50:50 composition of active (S)- and essentially inactive (R)-enantiomers, contributes to this difficulty. Each enantiomer can exhibit distinctive pharmacokinetics from its antipode and the nature of the chiral carbon allows all members the *in vivo* potential, although not necessarily the provision, to undergo an irreversible, apparently uni-directional (R)- to (S)- inversion. The extent of inversion is dependent upon drug, species and tissue preparation. The objectives of this work were to (1) examine the enantiomeric pharmacokinetics (PK) of a 2-APA not previously studied, (2) determine whether 2-APA inversion is a general process and (3) discern the site(s) of inversion. To this end, flurbiprofen (FL), was initially selected.

A stereospecific HPLC method was developed capable of quantifying FL enantiomers at concentrations normally encountered in human plasma. Derivatization with L-leucinamide allowed enantiomer separation on a reverse-phase column. Both man and the Sprague-Dawley (S-D) rat exhibited stereoselective FL enantiomer pharmacokinetics with non-detectable and minimal inversion, respectively, making the rat a suitable model for PK studies. An observation in man led to the demonstration in rats of an *in vivo* competitive interaction between FL enantiomers after racemate administration resulting in a nearly two-fold increase in (R)-FL clearance relative to single isomer administration. Administration of a dose range (1.25 to 40.0 mg/kg) of racemic FL revealed the

stereoselective enhancement of clearance of the (R)-enantiomer to be dose-dependent.

A 2-APA previously demonstrated to undergo a high degree of inversion in man, (R)-fenoprofen (FN), was selected for inversion studies. Using the male S-D rat three systems were examined, demonstrating significant inversion of (R)-FN (1) *in vivo* after oral and IV administration of (Rac)-FN and (R)-FN, (2) after (R)-FN perfusion of single-pass and recirculating isolated liver (extraction ratio=0.3) and (3) following a 2h incubation of (R)-FN with everted intestinal tissue. Maximal inversion by jejunum (serosal S:R ratio 2.2), relatively less by duodenum (1.2), ileum and colon, and total absence in the stomach was observed. Stereoselective glucuronidation in all GIT tissue segments as well as first-pass glucuronidation of both enantiomers following single-pass liver perfusion was observed. Therefore, in the male S-D rat, inversion of (R)-FN occurs presystemically (GIT and liver) and systemically (liver). These results, however, must be viewed with caution as they may not necessarily be extrapolated to other 2-APAs or species.

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List of Symbols and Abbreviations

AAT: Alanine aminotransferase (or ALT; glutamate pyruvate transaminase: GPT) (E.C. 2.6.1.2)

A_e: Cumulative amount of drug excreted unchanged in urine

ATA: Aspartate transaminase (or aspartate aminotransferase: AST; glutamate oxaloacetic transaminase: GOT) (E.C. 2.6.1.1)

AUC: Area under the curve

AUMC: Area under the first moment curve

2-APA: 2-Arylpropionic acid

β: Elimination rate constant

b.i.d.: twice a day

Cl_{TB}: Apparent total body clearance

Cl_R: Renal clearance

C_{max}: Maximum plasma concentration

CoA: Coenzyme A

Conc. Concentration

CV: Coefficient of variation

ER: Extraction ratio

F: Bioavailability

FL: Flurbiprofen

FN: Fenoprofen

GIT: Gastro-intestinal tract

HPLC: High-Performance Liquid Chromatography

HSA:	Human serum albumin
IB:	Ibuprofen
IPK:	Isolated perfused kidney
IS:	Internal standard
IV:	Intravenous
KT:	Ketoprofen
LTB_4:	Leukotriene B_4
nm:	nanometres
NA:	Naproxen
NSAID:	Non-steroidal anti-inflammatory drug
PD:	Pharmacodynamics
PK:	Pharmacokinetics
2-PPA:	2-Phenylpropionic acid (Hydratropic acid)
q.s.:	Make up to final volume
(R)-:	right (Latin: *Rectus*)
RA:	Rheumatoid arthritis
Rac-:	Racemate
(S)-:	left (Latin: *Sinister*)
S.D.:	Standard deviation
S-D:	Sprague-Dawley
SRS-A:	Slow reacting substance of anaphylaxis (*i.e.* LTB_4)
$t_{½}$:	Elimination half life
T_{max}:	Time of maximum plasma concentration

TEA: Triethylamine

TG: Triglyceride

t.i.d.: three times a day

UDPGT: Uridine diphosphate glucuronosyltransferase

UV: Ultraviolet

V_{dss}: Apparent volume of distribution at steady state

VLDL: Very-low-density lipoprotein

I. Introduction

Even with the most cursory perusal, it would be quickly apparent that the universe appears to be predominantly symmetrical--our own overall external appearance and those of most animals exhibit a bilateral anatomical symmetry, usually coupled with minor deviations from that symmetry. Even the plant life appears to exhibit an overall symmetry-from the spherical or radial symmetry of most fruits and vegetables to the bilateral symmetry of leaves. However, **inside** living things, at the molecular level, the reverse is found to be true: essentially all macromolecules and most carbon-based small molecules have a central-atom asymmetry. That is, about a centrally located atom are attached four different moieties with the overall 3-dimensional spatial arrangement resembling that of a slightly skewed tetrahedron. This very common internal arrangement of atoms is the source of the selectivity of one small molecule over another to preferentially bind with a protein receptor which eventually leads to some form of decided response. Even our senses, such as our perception of taste and smell, are governed by this selectivity or ***stereo***[1]-selectivity for receptors. These proteins, as with most in the body, can distinguish between two enantiomeric forms of a molecule, in this case either a flavour or an odorant. For example, a variety of tastes can be elicited by almost all amino acid enantiomers. Synthetically produced hydrophobic D-amino acids bring out a strong sweet taste with D-tryptophan and D-phenylalanine being 35 and 7 times as sweet as sucrose, respectively (Solms, 1969). In contrast, most of the natural hydrophobic L-amino acids have a bitter taste. L-glutamic acid has a sour taste while its

---

[1]stereo-from the Greek *stereós* meaning solid or three-dimensions.

sodium salt (monosodium L-glutamate, MSG) elicit the umami taste. Not only are free amino acids capable of eliciting a wide range of different taste perceptions, but many short chain peptides can also invoke a variety of tastes. Perhaps the best known of these is the sweet tasting aspartame (L-Aspartic acid-L-Phenylalanine-OMe). It is interesting to note that as free amino acids, L-aspartic acid and L-phenylalanine have sour and bitter tastes respectively (Kato *et al.* 1989). Our sense of smell in many cases is determined by a particular enantiomer. There are several examples where the two enantiomeric forms of a compound will have different odours: S-(+)-carvone gives the odour impression of caraway while its antipode that of spearmint (Russell and Hills, 1971). Also, the difference in the odours of oranges and lemons is caused by the R-(+) and S-(-)-enantiomers of limonene, respectively (Friedman and Miller, 1971). There are also examples where one enantiomer can have a very strong odour while its antipode is essentially odourless (e.g. (-)- and (+)-androsterone respectively)(Pickenhagen, 1989). Although there are sharp contrasts between enantiomers in the above examples, not all enantiomers possess different odours, for example, (+)- and (-)-camphor or (+)- and (-)-2-octanol (Friedman and Miller, 1971). Enantiomeric recognition is not limited to man, as bacterial chemotaxis depends upon the ability of the microbe to discriminate between enantiomers of sugars and amino acids (Adler, 1969).

When an asymmetric substance is administered as a racemate, the two enantiomers will come in contact with the inherent asymmetric structures present, be they small molecules or macromolecules. When the two enantiomers are scrutinized in three-dimensional space, they are fundamentally different compounds and the bodies response

with these compounds can take several possible courses. Pharmacokinetically, the two enantiomers may undergo a discrimination in absorption and/or disposition, observed either as inequalities in rate or extent of a process, a distinct preference of process affecting one enantiomer or diverse processes or dissimilar pathways taken by the enantiomers altogether. Pharmacodynamically, they may have identical qualitative and quantitative effects (e.g. halothane, propafenone and promethazine)(Ueda and Kamaya, 1984; Powell *et al.* 1988) or quantitative differences in potency, which could range from a small effectual difference to an almost total lack of efficacy by one enantiomer (e.g. warfarin, 2-APA NSAIDs)(Williams and Lee, 1985; Drayer, 1988; Hyneck *et al.* 1990). Or the two enantiomers may have qualitatively dissimilar effects which can be either in opposition (e.g. picenadol) or unrelated and diverse (e.g. ketamine) (cf. Drayer, 1988 and Jamali *et al.* 1989a). Furthermore, differences in the pharmacokinetics of the enantiomers can lead to stereoselectivity in the pharmacodynamics by determining the respective concentrations of the enantiomers at the receptor. For example, dispositional factors such as protein binding may cause the unbound fraction of one enantiomer to predominate, thereby conceivably allowing its effects and/or toxicity to dominate the observable response. Perhaps the more active enantiomer may have an equivalently larger clearance, thus equalizing the effects of the two enantiomers over time. Hence, any pharmacokinetic process that displays enantio-selectivity has the potential to alter the *in vivo* pharmacodynamics from that observed *in vitro*. In addition, through modification of physiological processes such as organ blood flow, the pharmacodynamics of the enantiomers may alter their own disposition kinetics. It is clear that the kinetics and

dynamics of enantiomers are intrinsically associated and the alteration of one may lead to a modification in the other.

Therefore, from the above discussions, it is clear that the universe is made up of two quite distinct, though non-mutually exclusive environments. These two milieus are termed **chiral**[2] and **achiral**[3]. Chirality exists from the macro-environments of spiral galaxies to the micro-environments of the simple chemical compounds of life such as those based on DNA made of 2-deoxyribose monomers of the D- (or R-) configuration and proteins made of amino acid monomers of only the L- (or usually S- , cysteine being the exception) configuration (Bonner, 1991). However, there are exceptions to the stereo-homogeneity of natural products, for example the antibiotic gramicidin S contains D-phenylalanine included in its cyclic decapeptide structure (Ulbricht, 1962). As enzymes are generally composed of only (S)-amino acids, stereospecificity in enzymatic catalysis is not unexpected. The term 'ambidextrous universe' has been used to describe the co-habitation of symmetric and asymmetric entities (Gardner, 1990).

1.1 A Brief History of Chirality

Although these chiral and achiral universes have been present since the origin of the cosmos, the scientific era of the 'ambidextrous universe' did not begin until 1815, when

---

[2]Chiral or asymmetric/dissymmetric--from the Greek *cheir* meaning hand, the term used by Lord Kelvin in 1894 (Gardner, 1990) to denote the property of a molecule (or any object) which has the feature of non-superimposability on its mirror image, like the left and right hands.

[3]Achiral or symmetric

Jean-Baptiste Biot discovered that sugars, tartaric acid and camphor rotated plane polarized light in solution, that is, optical activity. A young French chemist named Louis Pasteur learned that it was an intrinsic feature to the asymmetry of individual molecules and in 1848 having worked with tartaric acid, made his proposal to the Paris Academy of Sciences on the molecular asymmetry of organic molecules (Mason, 1988). Tartaric acid was found to have the same properties and composition as the acid produced from grape fermentation (then termed racemic acid), but one: fermentation-produced tartaric acid rotated polarized light whereas the synthetically produced acid did not. He discovered that synthetic tartaric acid was composed of an equal proportion of two mirror image crystalline forms[4]. By physically separating (with tweezers!) the two enantiomeric crystals of tartaric acid, he became the first to resolve a racemic compound into its individual optical antipodes. Pasteur also discovered that the left- and right-handed crystals had opposite effects on polarized light to which he proposed to be due to molecular asymmetry (Holmstedt, 1990). This property of optical isomerism--having the same molecular formula but different rotation of polarized light characteristics--occurred in other organic compounds. But only one form existed in natural products while with chemical synthesis both forms were produced in equal proportions so the substance exhibited no rotation of polarized light. Since compounds exist in their stereochemically pure state in nature, Pasteur deduced that organisms were selective toward isomers. Scarcely ten years later, August Kekulé discovered that carbon was tetravalent which

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[4] Today the term used for an equal proportion of two mirror images is racemate; from the Lat. *racemus* and Fr. *racemique*: a cluster of grapes.

helped Jacobus van't Hoff and Joseph LeBel, working independently, in 1874 to put forward the stereochemical theory of isomerism based on the concept of a non-planar tetravalent carbon. Van't Hoff specifically proposed that the four valence bonds of carbon had a spatial arrangement directed towards the four corners of a tetrahedron with carbon in the centre. With that, the architecture of organic compounds became three-dimensional. He defined a central carbon with four different substitutions as an *'asymmetrisch koolstof-atoom'* or asymmetric carbon, which could exist in two distinctly different non-superimposable forms (Weyer, 1974). This carbon, they proposed, was the cause of molecular asymmetry and therefore optical activity. Twenty years later one of Kekulé's former students, Emil Fischer suggested that the molecule had to fit a certain geometric space, *i.e.* his 'lock and key' paradigm (Mason, 1988).

Fischer's proposal provided the first understanding of the stereospecificity of biological action and the concept of receptors. Cushney, in 1908 showed a stereospecific difference existed between adrenaline enantiomers: the (-)-isomer had pressor activity while its antipode did not. 'The receptive substance', he concluded, '...is able to discriminate between enantiomers' and 'it may be inferred legitimately that the receptive bodies for these alkaloids are themselves optically active' (Cushney, 1908). Furthermore, it was suggested in 1933 by Easson and Stedman, that molecular dissymmetry and structure should not be differentiated only with respect to their contributions to physiological activity. Their three-point attachment hypothesis, proposed that if association of a xenobiotic with a receptor exhibits enantioselectivity, the interaction may involve three of the groups linked to the asymmetric centre. In other words,

enantioselectivity in activity arises from the differential affinity or attachment of a substrate for a receptor (Easson and Stedman, 1933; Weyer, 1974). The enantiomer which has the strongest receptor binding and vis-à-vis the greatest desired activity is termed the 'eutomer', while the 'distomer' is that enantiomer with lesser or no desired activity, but may exhibit untoward actions (Ariens, 1991).

Furthermore, although the enantiomers may exhibit considerably different affinities for the receptor, in the *in vivo* situation, each isomer, through competition, has the potential to affect the receptor binding of its antipode. As a consequence, this can lead to the racemate exhibiting lower activity relative to the single eutomer. This is observed with (+)-isoproterenol which lacks activity, competitively antagonizing the action of the active (-)-isomer, the end result being the racemate behaving as a partial agonist (Williams, 1989).

The principle of receptor association and enantioselectivity in pharmacodynamics can be extended to pharmacokinetics, where stereoselective parameters are determined by the interaction between chiral xenobiotics and endogenous macromolecules, which themselves are chiral. This will be discussed in greater detail in Section 1.3.

1.2 Structural Origins of Chirality

Molecular asymmetry or chirality, that is, the property of an object to display non-superimposability on its mirror image (Testa, 1982), can arise via a number of three-dimensional possibilities in molecules (Matlin, 1989). These include:

a) one or more centres of chirality, where four different substituents, which can

include an unshared electron pair serving as one of the coordinates, are bonded to a central tetrahedral atom--usually carbon, but may also be phosphorous, silica, germanium, arsenic, sulphur, or nitrogen. While a quaternary nitrogen is chiral and configurationally stable, it is the nature of the substituents and thus the energy barrier for pyramidal inversion that determines the configurational stability of tricoordinate nitrogen enantiomers. In addition, chiral coordinate organometallic complexes (Sokolov, 1990; Noyori, 1989) also exist with species such as chromium (Hegedus *et al.* 1992) and iron (Stolz *et al.* 1992) forming the central atom.

Whereas a chiral centre is the most common basis of asymmetry, the remaining five structural examples do not depend on a single centre but rather the overall spatial relationship gives rise to the asymmetry, such as:

b) planar chirality (e.g. trans-cycloalkenes: paracyclophanes) where the presence of a chiral plane causes asymmetry with respect to that plane.

c) axial chirality (e.g. spiranes with cyclic skeleton, allenes and biphenyls)

d) helicity

e) torsional chirality (e.g. torsion about double or single bonds) or

f) topological asymmetry (e.g. catenanes or chiral cages).

1.2.1 Sources of Chiral Compounds: *In Vitro*

Chiral pharmaceuticals, both natural/semi-synthetic and synthetic, have been estimated to make up between 50 to 80% of the drugs marketed over the last decade (Mason, 1984; Simonyi, 1984; Noctor *et al.* 1986; Ariens *et al.* 1988). Generally there

are four methods of obtaining chiral compounds (Beld and Zwanenburg, 1986):

- Isolation from material of plant, animal or microbial origin as most naturally occurring medicines (e.g. (-)-morphine from *Papaver somniferum* and (+)-digitoxin from *Digitalis purpurea*) exist as the single isomer. Today many of the natural products are synthetically produced.
- Chemical or enzymatic transformations of chiral moieties into other chiral compounds.
- Asymmetric synthesis and
- Resolutions of racemic mixtures or compounds: a racemic mixture being a 50:50 combination of two enantiomers where each individual crystal is optically pure, and whereas a racemic compound is also a 50:50 combination, in contrast to a mixture, all crystals have an equal quantity of both enantiomers per unit crystal cell (Dwivedi *et al.* 1990; Jacques, 1981).

1.2.2 Sources of Chiral Compounds: *In Vivo*

Phase I and II enzymatic metabolism is predominately the source of *in vivo* chiral compound formation. Briefly, phase I metabolism may be *prochiral* whereby a compound can acquire chirality *via* metabolism either remote to the prochiral centre (e.g. phenytoin) or the prochiral centre itself (e.g. fenofibric acid) (Caldwell *et al.* 1988b). Also, rare reactions such as enantiomeric racemization, epimerization or inversion can be thought of as chiral-chiral metabolism (Hutt and Caldwell, 1984; Aso *et al.* 1990). Phase II metabolism can introduce a second chiral centre by conjugation of a xenobiotic

with endogenous compounds such as β-D-glucuronic acid or glutathione (Caldwell *et al.* 1988b; Mulder *et al.* 1991).

1.3 Clinical Administration: Racemate or Single Enantiomer?

As previously discussed, in an achiral milieu most of the physical properties of an enantiomeric pair are identical[5], but exposure to a chiral environment containing chiral small molecules and biological macromolecules, results in the formation of a pair of temporal diastereomers that differ in their physical properties. These differences may be observed in any or all of the pharmacokinetic processes such as absorption, distribution, metabolism and excretion. Any overall perception of a pharmacokinetic or pharmacodynamic difference between enantiomers, that is, the degree of stereoselectivity, is dependent on whether the molecular difference(s) translate into whole organ differences and ultimately into macroscopic or whole body system differences (Levy and Boddy, 1991).

Therefore, as each isomer of an enantiomeric pair may have distinct kinetics and/or dynamics, the clinical use of racemates may lead to a perplexing complexity, including: erroneous models of pharmacokinetic behaviour (Evans *et al.* 1988), misinterpretation of drug-drug interactions (e.g. warfarin-phenylbutazone, Aggeler *et al.* 1967; Banfield *et al.* 1983) and/or to a bewildering array of pharmacodynamic possibilities dependent

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[5]Generally, with the exception of the ability of the enantiomers to rotate plane-polarized light to an equal degree but in opposite directions, the physical properties of an enantiomeric pair are identical.

on the qualitative and quantitative pharmacological relationship between the enantiomers (Hyneck *et al.* 1990; Powell *et al.* 1988). Ultimately, this can lead to difficulties in drug regimen prediction resulting in a lost potential to manipulate the therapeutic endpoint.

Due to the several documented differences in efficacy, toxicity and/or pharmacokinetics of individual enantiomers (Drayer, 1988; Jamali *et al.* 1989a; Tucker and Lennard, 1990), concerns of a regulatory, ethical and therapeutic nature have arisen with respect to racemic therapeutics. Essentially, can one in good conscience administer, or allow to be administered, to patients, a medicinal agent with either the suspicion or knowledge, that from 50% (if only one enantiomeric pair exists) up to 75% (if two chiral centres exist where only one of the four possible entities is active) of the drug entity *may* be clinically inactive but *may* contribute to overall toxicity or *may* contribute to a decrease in the efficacy of the active enantiomer, either by competing for the receptor or qualitatively functioning as an antagonist. The inclusion of the above italicized auxiliary verb is obligatory because quite simply, the vast majority of clinically administered racemates have yet to be characterized. That is, nescience regarding the pharmacokinetics of individual enantiomers and/or the effect of the distomer on the overall pharmaco-therapeutics of a drug is, unfortunately, the norm.

Recent articles have discussed the value and position of optical rotation in policy recommendations in drug standards (Chafetz, 1991) as well as the position of pharmaceutical firms regarding racemates (Pharmaceutical Manufacturers Association, 1990). In addition, the perspective of the FDA in the US was recently outlined by De Camp (1989). At present, the Health Protection Branch (HPB) in Canada does not

require specific stereochemical information with new drug submissions (Rosier and Demoen, 1990). Interestingly, in the document "Chemistry and Manufacturing Guidelines Preclinical New Drug Submissions" released by the HPB (1984) requires a distinction made between potential impurities and actual impurities, although no mention is made regarding the inactive isomer(s) present in racemic drug products, *i.e.* 'isomeric ballast' a term first used by Ariens (1984).

1.4 The 2-Arylpropionic Acid Class

Generally, a relationship between therapeutic response and plasma concentrations of non-steroidal anti-inflammatory drugs (NSAIDs), especially within the 2-APA class, has in most cases been difficult to achieve and explanations have been wide ranging (Brune, 1985; Day and Brooks, 1987; Day *et al.* 1988; Simkin, 1988). As a class, the 2-APAs are unique among the NSAIDs[6] as each member contains a chiral centre: an asymmetric carbon atom located α to the carboxylic acid moiety. With the single exception of naproxen, all the 2-APAs marketed have been administered as racemates. The apparent lack of relationship between dose, concentration and response of the clinically administered 2-APAs *may in part* be the consequence of their consumption as racemates. This hypothesis may have some merit as among the large number of 2-APA NSAIDs administered clinically, only naproxen (Day *et al.* 1982; Dunagan *et al.* 1988) and carprofen (Furst *et al.* 1988) have demonstrated a dose-response relationship. Table 1.1

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[6]Other individual NSAIDs contain a chiral centre: 1) sulindac which has a chiral sulphur atom and 2) several members of another arylalkanoic class, e.g. etodolac and ketorolac.

lists the world-wide investigational and commercially available 2-APAs, both past and present.

In order to correlate concentration with effect, measurement of the active component, be it the parent drug or a metabolite, should intuitively provide the best relationship. For agents that are administered as a racemate, where the two enantiomers may have either qualitative or quantitative differences, measurement of the eutomer is clearly indicated as measurement of the total (*i.e.* sum of the enantiomers) could potentially lead to erroneous conclusions.

As will be discussed in greater detail in section 1.4.1, it is generally believed that with all members of the 2-APA class, it is the S- or (+)-enantiomer[7] which possesses the anti-inflammatory activity of the racemate, with the R- or (-)-enantiomer (Flippen and Gilardi, 1975) essentially devoid of activity (Hutt and Caldwell, 1984). Furthermore, the (R)-enantiomer of some of the 2-APAs may undergo a unique *in vivo* irreversible inversion to the (S)-enantiomer. The inversion, however, is not a universal occurrence and is dependent on both drug and species. Therefore, depending on whether an agent undergoes inversion, the (R)-enantiomer cannot be presumed inactive and perhaps may be considered a pro-drug. Furthermore,the (R)-enantiomer may contribute to the toxicity as will be discussed later.

In view of the unique attributes of the 2-APAs discussed above, it is not surprising that there has been difficulty in exposing a concentration-effect relationship.

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[7]Absolute configuration of enantiomers, if known, designated (R)- or (S)- (Cahn *et al.* 1956) and if unknown by the direction, (+):right or (-):left, of optical rotation.

Table 1.1 Investigational, Discontinued and Commercially Available 2-Arylpropionic Acid NSAIDs[1]

Class Members	Selected Trade Name(s)
alminoprofen	
benoxaprofen[2]	Oraflex[2]
carprofen	
cicloprofen	
fenoprofen[3]	Nalfon, Fenopron, Nalgesic
flunoxaprofen[4b]	
flurbiprofen[3]	Froben, Ansaid
furprofen	
ibuprofen[3]	Advil, Apo-Ibuprofen, Motrin, Novoprofen
indoprofen	
ketoprofen[3]	Orudis, Rhodis, Alrheumat
loxoprofen[5]	
naproxen[3],[4a]	Naprosyn, Apo-naproxen, Novonaprox
naproxen sodium[3,4a]	Anaprox, Novonaprox Sodium
pirprofen	
pranoprofen	
suprofen	
thioxaprofen	
tiaprofenic acid[3]	Surgam
ximoprofen	

[1] Information collected from: Muller *et al.* 1990b; Arima, 1990; British Pharmacop. 1988; Compend. Pharmaceut. Special. 1992; U.S. Pharmacop. XV, Diekmann *et al.* 1979, Lan *et al.* 1976, Nagashima *et al.* 1984, and Muller, 1990

[2] Withdrawn from market (Marshall 1985).

[3] Available in Canada.

[4a] Marketed as single (S)-enantiomer.

[4b] Undergoing clinical trials as (S)-enantiomer in Italy (Priaxim®).

[5] Contains two chiral centres, therefore is marketed as two enantiomeric pairs (Loxonin®)

1.4.1 Anti-inflammatory Activity and Mechanism(s) of Action

Like other NSAIDs, the 2-APAs interact with a variety of cellular systems including those presently thought responsible for the major mechanisms of action of NSAIDs: prostaglandin synthesis inhibition (Vane and Botting, 1990) and neutrophil inactivation *via* interference with stimulus-response coupling (Weissmann 1987; Abramson and Weissmann, 1989; Abramson *et al.* 1991).

It is the measurement of PG synthesis inhibition that is most commonly used to determine the relative anti-inflammatory potency of the enantiomers. The individual (R)- and (S)-enantiomers are exposed to a variety of *in vitro* tissue preparations (e.g. bovine seminal vesicles and/or platelet aggregation inhibition) specifically designed to measure, directly or indirectly, the inhibition of prostaglandin synthesis. The resulting (S/R) or 'eudismic ratio'[8], is used to roughly quantitate the relative enantiomeric potency, a supposed measure of the ability of the various agents to inhibit the inflammatory processes. For example, Adams *et al.* (1976), using a bovine seminal vesicle microsomal preparation, showed that (S)-ibuprofen was 160-fold more active in prostaglandin synthesis inhibition than its antipode. Using human platelets as the model, Rubin *et al.* (1985) found (S)-fenoprofen to be 35-fold more potent than the (R)-isomer. Perhaps the largest *in vitro* eudismic ratio observed occurs with the antagonism of SRS-A by flurbiprofen where the difference in potency between the (S)- and (R)-enantiomers is 878-

[8]Eudismic ratio: the ratio of the activities (dynamics) of the eutomer (i.e. the enantiomer possessing either all or the majority of the desired activity under discussion) and distomer (i.e. the enantiomer which either lacks or has the lesser/diminished activity currently under discussion) (Lehmann *et al.* 1976; Ariens, 1991).

fold.

In addition to *in vitro* studies, several *in vivo* animal models (e.g. carrageenin induced arthritis) are employed to determine the relative enantiomeric anti-inflammatory potency. Surprisingly, when the two enantiomers of fenoprofen (discussed above) were examined *in vivo*, their potencies were found to be identical (Rubin *et al.* 1985). The enantiomers of ibuprofen (Adams *et al.* 1976) were similarly found to be of almost identical potency with a eudismic ratio of 1.3. In fact, when comparisons are made the *in vitro* and *in vivo* eudismic ratios of all the 2-APAs studied, a disparity was observed ranging from the minor (carprofen: approx. 23 *vs.* 15 respectively; Gaut *et al.* 1975) to the major (e.g. flurbiprofen 878 *vs.* 8; Greig and Griffen, 1975). Initially, the discrepant findings between the *in vitro* and *in vivo* extent of stereoselectivity were regarded to be almost entirely the result of the *in vivo* uni-directional inversion (c.f. Lee *et al.* 1985).

Invariably, based on the results of the above studies, any comparisons between the *in vitro* activity of the individual 2-APA enantiomers report only the (S)-enantiomer as active. This commonly accepted conclusion is based on the general belief that the major mechanism of action of the NSAIDs in the treatment of inflammation is *via* inhibition of prostaglandin synthesis. Accordingly, the majority of published studies dealing with the anti-inflammatory activity of the 2-APAs have focused primarily on the effects of the racemate on prostaglandin synthetase inhibition. Recently however, increasing evidence suggests that inhibition of stimulus-response coupling in the neutrophil may be the major mechanism or at least an important additional mechanism by which NSAIDs exert their anti-inflammatory activity (Abramson and Weissmann, 1989; Kitsis and Weissman,

1991).

Not surprisingly, recent studies have become increasingly involved in the role of the single enantiomer on the inflammatory processes. One such study involved the interesting property of chemiluminescence whereby neutrophils emit a burst of light upon ingestion of opsonized zymosan[9]. In a brief report, Cassone (1988) examined whether this phenomena had a potential usefulness in the determination of non-steroidal anti-inflammatory activity of a few selected 2-APAs. Using (S)-, (R)- and (Rac)-ibuprofen, it was found that phagocytic activity was inhibited approximately 97, 92 and 85%, respectively. Although pertinent information is lacking (e.g. dosages and whether equimolar drug concentrations were used), two inferences can be made. First, the activity of each single enantiomer exceeds that of the racemate, indicative of a possible enantiomeric interaction with racemate administration and second, although neutrophils are assumed to lack metabolic capability to carry out inversion, the efficacy of the (R)-enantiomer approached that of the (S)-enantiomer. This suggests that 2-APA involvement in neutrophil inactivation may be non-stereoselective, diametric to prostaglandin synthetase inhibition. Furthermore, these findings may, in part, explain the apparent discrepancy between the (S/R) eudismic ratio of the *in vitro* and *in vivo* studies. One explanation for the discrepancy involves the supposition that in an isolated tissue, any action of the 2-APAs will affect only prostaglandin synthetase, as neutrophil activity is not considered. In fact, any past studies using isolated tissue to examine the efficacy of

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[9]Opsonized zymosan: zymosan (an insoluble polysaccharide found in the cell wall of certain yeasts) treated to increase the susceptibility to ingestion by phagocytes.

the 2-APAs are singularly based on the accepted premise that the PGs are the major instigators of inflammation. Therefore, most *in vitro* assays only measure the inhibition of PG synthesis (Hutt and Caldwell, 1984) and are not designed to measure the activity of the neutrophil, the other possible cause of inflammation.

Nevertheless, it is a widely held belief the *in vivo* (R) to (S) inversion, a pathway apparently considered by some to be absent in isolated tissue, explains the discrepancy between the *in vivo* and *in vitro* eudismic ratios (Caldwell, 1990). In other words, *in vivo*, the (R)-isomer of all the 2-APAs act as a pro-drug, and any observed inhibition of inflammation was due to the newly-formed (S)-enantiomer (see section 1.4.3).

1.4.2 Stereoselective Pharmacokinetics of the 2-Arylpropionic Acids

1.4.2.1 Absorption

As the physical properties that control passive absorption through a lipid membrane (partition coefficient, pKa) of an enantiomeric pair are by definition identical, absorption from the gastro-intestinal tract can be expected to be non-stereoselective, although enantioselective interaction with membrane proteins is a possibility. Experimentally in humans, the C_{max} and T_{max} values for the 2-APA enantiomeric pairs of tiaprofenic acid (Singh *et al.* 1986), flurbiprofen (Jamali *et al.* 1988b), and ketoprofen (Foster *et al.* 1988a and b) are identical, confirming that it is unlikely there are stereoselective differences in absorption.

In man, a few agents display enantiomeric similarities in T_{max} but differences in the C_{max}--for example, flunoxaprofen (Palatini *et al.* 1988), indoprofen (Tamassia *et al.* 1984)

and ibuprofen (Jamali *et al.* 1988a). After oral administration of ibuprofen, the observation that the C_{max} (S/R) ratio was not unity, and the ratio increased proportional to the GIT residency of the dosage form, lead Jamali *et al.* (1988a) to speculate the GIT as a major site for inversion. Other workers have shown a similar trend of increasing C_{max} (S/R) ratio with T_{max} (Borin *et al.* 1988). After racemate administration of ibuprofen to humans, the T_{max} for the enantiomers were found to be identical, but the mean C_{max} for the (S)-isomer exceeded that of the (R)-isomer (Geisslinger *et al.* 1989 and 1990). Avgerinos and Hutt (1990) had similar findings with ibuprofen--near identical enantiomeric T_{max} values and a C_{max} (S/R) ratio of 1.4. Recently, using isolated human intestinal tissue, a significant degree of inversion was observed confirming the GIT as a site of inversion (Jamali *et al.* 1991). Campbell (1990a) states that absorption is stereoselective if there is gut or gut wall metabolism. However, if absorption is enantioselective, a difference between the absorption rate constants of the enantiomers would be expected. This would be seen as either an active absorption or a selective promotion of intestinal permeability, as suggested with the (-)-enantiomer of terbutaline, enhancing the absorption of its antipode (Borgstrom *et al.* 1989). If a 2-APA undergoes intestinal inversion upon oral absorption, any remaining un-inverted (R)-isomer and newly formed (S)-isomer will continue to have identical K_a's, regardless of the extent of inversion process. Therefore, the activity in the gut would more appropriately be termed stereoselective *first-pass metabolism*, not absorption. A more detailed discussion on the location of inversion will be given in Section 1.4.3.2.

Furthermore, any enantiomeric absorption differences observed after oral

administration of a solid dosage form but absent with a solution, consideration must be given to the possibility of stereoselective release from the dosage form matrix. Since many tablet excipients (e.g. cellulose derivatives and investigational cyclodextrin) have chiral centres, the formation of temporal diastereomers between the drug and matrix is a possibility, as observed with propranolol (Duddu *et al.* 1991).

1.4.2.2 Distribution: Plasma Protein Binding and Volume of Distribution

It has been suggested that the single most important factor determining the observed distribution and elimination pharmacokinetics of the 2-APAs is their plasma protein binding characteristics (Verbeek *et al.* 1983). Of the three major plasma proteins involved in drug binding: albumin, α_1-acid glycoprotein and the lipoproteins, the 2-APAs bind preferentially to albumin, specifically at site I (warfarin/azapropazone site)(Müller, 1988) due to their acidic nature (pKa range: $\approx$ 3.5-5). As a result of the extensive degree of binding to plasma proteins ($>$ 99%), the 2-APAs are characterized by a relatively low volume of distribution ($<$ 0.2 L/kg) and Cl_{TB} ($<$ 100 mL/min.) (Verbeeck *et al.* 1983). The majority of the 2-APAs are bound stereoselectively (carprofen, Iwakawa *et al.* 1990; flurbiprofen, Knadler *et al.* 1989; ibuprofen, Evans *et al.* 1989; ketoprofen, Rendic *et al.* 1980). However, Perrin *et al.* (1973) reported fenoprofen as the first of two 2-APAs which do not display differential enantiomer binding to serum proteins. For the second case, Otagiri *et al.* (1989) used equilibrium dialysis as well as several spectroscopic techniques to examine the interaction of pirprofen with purified HSA and found that the binding of pirprofen is apparently non-stereoselective. However,

that study used abnormally low albumin concentrations relative to normal plasma making the drug-to-albumin ratio higher than would be seen therapeutically, resulting in a possible masking of any stereoselectivity. A more recent report which utilized similar spectroscopic techniques as well as HPLC, found a slight stereoselectivity in specific binding of pirprofen at expected therapeutic concentrations, with the (R)-enantiomer showing a slightly larger association constant to HSA (Oravcová *et al.* 1991).

The significance of stereoselective binding is two fold: for low extraction drugs such as the 2-APAs, the clearance of each enantiomer is proportional to its respective unbound fraction in plasma, therefore the least bound will have more free drug in plasma and a higher clearance, but as reported for disopyramide (Lima *et al.* 1987), this is not always the case. Also, the importance of the relative association constants in competitive interaction is significant in determining the overall displacement, thereby determining the respective free plasma concentrations--the species associated with pharmacological activity.

Competitive enantiomer-enantiomer protein binding has been suggested for several of the arylpropionic acids. In man, the disposition of (R)-ibuprofen given singly was noted to have a larger AUC than when administered in the racemate (Lee *et al.* 1985). A perturbation in protein binding as the result of an enantiomer-enantiomer interaction was suggested as the cause of the alteration. Mutual enantiomeric competition in protein binding between the enantiomers of 2-phenylpropionic acid (2-PPA) was observed *in vitro* using rabbit plasma (Jones *et al.* 1986). Interestingly, although the (R)-enantiomer was not examined, the (S)-isomer of indoprofen administered either singly or in the

racemate, failed to exhibit any change in disposition kinetics (Tamassia *et al.* 1984). Therefore it is apparent that existence of competitive interaction cannot be assumed.

1.4.2.3 Distribution: Triglyceride and Adipose Tissue Uptake

The incorporation of a wide variety of xenobiotics into triacylglycerols by covalently bonding to the glycerol backbone in positions normally occupied by 16- and 18-carbon fatty acids has been reported by several workers (cf. Fears, 1985; Dodds, 1991). Using rat liver slices, Fears *et al.* (1978) were the first to show that the 2-arylpropionic acid derivatives can act as substrates for this reaction, with the subsequent formation of unusual glycerolipids. They found that the relative incorporation into the glycerol backbone was: fenoprofen > ibuprofen > ketoprofen, with flurbiprofen failing to be assimilated into glycerol. In addition, it was revealed that these 2-APAs inhibited lipid synthesis in the same relative order as their glycerol incorporation. They speculated that for these xenobiotic conjugates to be formed, a prior formation of the acyl-CoA thioester was necessary. The formed acyl-CoA has the potential to undergo at least four possible metabolic pathways: (i) transport across the inner mitochondrial membrane as the acyl-carnitine for subsequent β-oxidation, (ii) incorporation into lipids as outlined above (iii) chiral inversion (discussed in detail section 1.4.3) and (iv) as a precursor in amino acid conjugation (Fears, 1985). As the 2-APAs are not known to undergo phase II amino acid conjugation, it has been speculated that a portion of the formed reactive acyl-CoAs are thus unable to be diverted and therefore proceed via other pathways, including glycerolipid formation. (Fears, 1985).

Following the initial finding by Fears *et al.* (1978), there have been several subsequent reports confirming the existence of the acyl-CoA thioesters and detailing the incorporation of some 2-APAs into liver and adipose tissue (Williams *et al.* 1986; Sallustio *et al.* 1987; Sallustio *et al.* 1988a; Moorhouse *et al.* 1991). Perhaps the most important finding from the latter studies was the discovery of the relative incorporation of the enantiomers into tissue: only the (R)-enantiomers of ibuprofen and fenoprofen were found to be capable of forming the hybrid triglycerides and subsequently only these (R)-isomer-containing TGs were able to be incorporated into tissue. The (S)-enantiomer was excluded from this process, rendering the uptake stereospecific. For example, only the (R)-enantiomer of ibuprofen was detected in rat adipose (Williams *et al.* 1986) and liver tissue (Moorhouse *et al.* 1991), as well as only (R)-fenoprofen in the rat hepatocytes and adipocytes (Sallustio *et al.* 1987; Sallustio *et al.* 1988a).

Fears and Richards (1981) were able to show a relative correlation between the ability of racemic fenoprofen and ibuprofen to form substituted glycerolipids and their ability to inhibit the synthesis of fatty acids and sterols. They suggested that the hypolipidemic effect by these agents is the result of depletion of CoA pools via CoA thioester formation and subsequent incorporation into glycerolipids. Recently, Sallustio *et al.* (1990) found that the previously observed inhibition of endogenous triglyceride formation by racemic fenoprofen and ibuprofen (Fears *et al.* 1978) is also stereospecific for the (R)-isomer at therapeutically relevant concentrations. The proposed mechanism of action is either competition of the (R)-enantiomer with the fatty acids for thioester formation or competition of the formed acyl-CoAs for glycerol incorporation (Fears *et*

al. 1978; Sallustio *et al.* 1990).

1.4.2.4 Elimination

The principle routes of elimination of the 2-APAs are Phase I oxidation and Phase II conjugation-with acyl glucuronide formation on the carboxylic acid moiety to give the primary urinary metabolite (Caldwell *et al.* 1988). The *in vivo* carboxylic acid glucuronidation is best described by reversible metabolism as the formed glucuronides are hydrolysable by esterase catalyzed hydrolysis (Spahn-Langguth and Benet, 1992). This process has been described as a 'futile cycle' of conjugation-deconjugation (Meffin *et al.* 1983; Rowe and Meffin, 1984). Examining the hydrolysis process *in vitro*, Nakamura and Yamaguchi (1987) using 2-PPA as substrate, have shown the process to be stereoselective, preferring the (R)-enantiomer.

The resultant glucuronide conjugates are diastereomers as glucuronic acid (β-D-configuration) is chiral. Whether 2-APA glucuronide formation is stereoselective is dependent on both species and drug (Caldwell *et al.* 1988). Due to both the labile nature of the glucuronides and the possibility of β-glucuronidase resistant conjugates (*via* acyl migration), caution must be exercised when reviewing enantiomer-glucuronide quantitation from the literature. In cases where the glucuronides were collected from healthy volunteers and stabilized prior to quantitation, it is clear that some 2-APAs exhibit little stereoselectivity in the amount excreted in the urine (carprofen Ae%: 77.3 and 72.3 for S and R respectively, Spahn *et al.* 1989); others exhibit moderate stereoselectivity in the elderly (ketoprofen Ae%: 69.8 (S) and 53.0 (R), Foster *et al.*

1988b), while other exhibit marked stereoselectivity (fenoprofen Ae%: 48.5 (S) and 3.3 (R), Spahn-Langguth and Benet, 1992). Other examples in man (which did not stabilize the glucuronides) include ibuprofen where there was preferential (S)-isomer glucuronidation as evidenced by the lower amount of its antipode glucuronide recovered. However, there are problems associated with this reasoning as will be discussed below.

The urinary clearance of a metabolite can involve up to four processes: (i) formation of the metabolite, (ii) conjugation of the metabolite, (iii) hydrolysis of the conjugate, and/or (iv) renal excretion of the conjugated or free metabolite. Any (or all) of these processes have the potential to be stereoselective. Therefore, any stereoselectivity observed in the Ae could have arisen from any of the above four processes. In fact, whether these processes can be discriminated or observed overall will depend essentially on the magnitude and/or predominant isomer resulting from each process (cf., Levy and Boddy, 1991). Therefore, equal diastereomer glucuronide recovery in the urine is not necessarily indicative of non-stereoselectivity, as a balancing of the above processes may result in equal proportions of the diastereomeric conjugates in urine.

Several workers have attempted to discern each process separately by utilizing various *in vitro* systems. It has been found that stereoselective glucuronidation is dependent on both the preparation (*i.e.* enzyme source) and the substrate. Using rat liver microsomes, the (S)-isomer of naproxen was preferentially glucuronidated while the (R)-isomer of benoxaprofen, carprofen, cicloprofen, flunoxaprofen, flurbiprofen and indoprofen showed a greater degree of glucuronidation over their antipodes. Interestingly,

ketoprofen enantiomers failed to display a preference (Spahn, 1988).

The observed 2-fold difference in clearance of the (R)-enantiomer of carprofen in the rat is attributed to that isomer's faster biliary excretion (Kemmerer *et al.* 1979). Any apparent stereoselectivity in biliary excretion must be viewed with caution, as it may simply be a reflection of that seen in plasma or it may result as a compensation of a stereoselective renal excretion rather than a hepato-biliary carrier-mediated stereoselective transport process.

1.4.3 Stereoselective pharmacodynamic and toxicologic considerations of the 2-arylpropionic acids

An informed decision regarding the use of either the racemate or single enantiomer must involve an unbiased consideration of the pharmacodynamic, toxicological and pharmacokinetic arguments of each 2-APA on an individual basis. Ideally, this should involve a three-way comparative examination of the racemate and each individual enantiomer. However, as discussed below, well controlled trials to address this question are lacking.

1.4.3.1 Pharmacodynamic Aspects

As discussed previously in Section 1.4.1, *in vitro* studies have shown the anti-inflammatory activity of the 2-APAs resides almost entirely with the (S)-enantiomer (Hutt and Caldwell, 1983, Hutt and Caldwell 1984). The anti-inflammatory activity in the (R)-enantiomer, depending on the *in vitro* test employed, can range from 0.11%, 0.63%,

2.86% to 4.35% (flurbiprofen, Greig and Griggen, 1975; ibuprofen, Adams *et al.* 1976; fenoprofen, Rubin *et al.* 1985; and carprofen, Gaut *et al.* 1975, respectively), of the (S)-enantiomer. This being the case, what is the justification in retaining of the (R)-enantiomer in the dosage form other than the increase development costs involved in enantio-separation and/or asymmetric synthesis. As several of these agents are able to undergo uni-directional inversion, it could be argued that the (R)-enantiomer serves as a pro-drug and there would be little advantage in the administration of the single (S)-enantiomer. Justification of this argument is only plausible if the extent of inversion is (a) substantial (b) predictable and (c) the time course to completion is reasonably rapid. Otherwise unknown variables are introduced making dosage regimen predictions difficult.

From this perspective, an examination of the pharmacodynamic evidence, both *in vivo* and *in vitro*, for the administration of the single enantiomer is necessary. Toxicological considerations will be dealt with in the next section.

The first clinical report to comparatively examine whether single (S)-enantiomer administration offered any advantages over the racemate used flurbiprofen as the test drug (Sunshine *et al.* 1987). Using subjective scoring in post-episiotomy pain, the mean of two dosage levels of the racemate were compared with the mean of two dosages of the (S)-enantiomer, administered at one-half the racemate dose. Interestingly, although the two groups received equimolar dosages of the (S)-enantiomer, administration of the single (S)-enantiomer provided analgesia superior to the racemate group, indicative that with the racemate an interaction was occurring, perhaps either at the pharmacodynamic receptor level or at the level of the various pharmacokinetic processes. Nevertheless,

this was the first report whose findings preferentially supported singular enantiomer administration.

The first clinical experience with rheumatoid arthritis and the use of a single enantiomer, in this case (S)-ibuprofen, was the subject of two recent reports (Geisslinger *et al.* 1989; Geisslinger *et al.* 1990). These studies found that between 500 mg b.i.d. or t.i.d. was sufficient to provide symptomatic relief to the patients. Unfortunately, neither study compared the dosage of (S)-isomer administered or the resulting efficacy with that of the racemate, thereby providing no comparative pharmacodynamic information on which to base a conclusion.

However, the same group (Stock *et al.* 1991) has recently reported a double-blind randomized study using fifty RA patients to compare the efficacy of (S)-ibuprofen (mean dosage: 1220 $\pm$ 60 mg/day) with that of the racemate (mean dosage: 1870 $\pm$ 140 mg/day). Interestingly, using a visual analogue pain scale, the racemate group had statistically significant better overall analgesic effect ratings than the single isomer. Whether this is due to an inequivalency among the two dosages or another reason is not clear.

Recently, Cox *et al.* (1991) reported administering 300 mg/day of (Rac)-flurbiprofen to twenty RA patients in an attempt to determine if a correlation existed between the total (S)-enantiomer plasma concentration and response. They found no significant correlations between total (S)-FL concentrations and a variety of subjective and objective outcome measurements. There are at least two components of this study which causes their results and subsequent conclusions to be suspect. First, it is a widely held belief that it is the

free or unbound drug, not the total drug concentration that is responsible for the efficacy observed and which normally provides the best correlations with response. Second, several classes of drugs have demonstrated enantiomer-enantiomer interaction when administered as the racemate (Murphy *et al.* 1976). Therefore, if a similar interaction occurs in man, then any attempt at determining a correlation between concentration and response may prove futile.

Using the adjuvant arthritic Lewis rat as a model, Jamali *et al.* (1989) administered (Rac)-ketoprofen at five different dosages in an effort to correlate any reduction in paw volume with plasma (S)-KT concentrations. Although the reported paw volume standard deviations were large, a clear concentration-response relationship ending in a plateau was observed.

Recently, when examining the *in vivo* differential analgesic efficacy of flurbiprofen enantiomers, Brune *et al.* (1991) found little difference between the two enantiomers. These authors suggest a non-PG synthetase-involved method of analgesia.

In addition to arthritis, other uses as well as other routes of administration of the 2-APAs include:

(a): pharmacokinetic investigation into the topical (buccal) administration of (S)- and (Rac)-flurbiprofen as a toothpaste or mouthwash for the inhibition of bone loss secondary to periodontitis (Wechter *et al.* 1991).

(b): investigation into the use of topical administration of (S)-ibuprofen as an alternative to topical corticosteroid therapy in ocular inflammation (Tilden *et al.* 1990).

(c): as a pre-medication for the inhibition of intraoperative miosis.

There are cases in the literature (Tjebbes *et al.* 1990) where the single (S)-enantiomer is tested for a specific effect, unfortunately these studies fail to carry out a comparative study with either the antipode or the racemate.

1.4.3.2 Toxicological Aspects

Although most discussions regarding the 2-APAs have centered on the use of the (S)-enantiomer versus the racemate, one report has suggested the (R)-enantiomer, *not* the active (S)-enantiomer be used (Williams and Day, 1988). The reason being that because of inversion, the (R)-isomer functions as a prodrug. In addition, the (R)-isomer may have less gastro-intestinal side effects (Williams and Day, 1988). Theoretically, this may be true. It is well known that endogenous and exogenous PG's have a cytoprotective action on the GIT and as it is generally accepted that the (S)-enantiomer alone has the ability to inhibit PG synthatase (*vida supra*). Therefore, if the ulcerogenic activity of NSAIDs is the result of PG synthatase inhibition, and inhibition eliminates the cytoprotective quality of the PG's, then vis-a-vis the endogenous cytoprotective effect of the PGs may be preserved upon administration of the single (R)-enantiomer.

Therefore the premise that the (R)-enantiomer would be less ulcerogenic makes four assumptions: (i) that minimal, if any, inversion occurs in the GIT, (ii) if GIT inversion does occur, any (S)-isomer formed would not have extensive contact with the mucosa, in other words, inversion would have to occur post-epithelial (*i.e.* lamina propria, sub-mucosa, muscularis mucosa/externa or serosa) and sink conditions would exist to minimize back-diffusion and mucosal contact, (iii) minimal biliary excretion of formed

(S)-isomer and (iv) that little, if any post-absorption or 'second-pass' metabolism takes place in the GIT, as reported with the digitalis glycosides (Lauterbach, 1981). Otherwise there would be little advantage in attempting to minimize gastropathy by preferentially administering the (R)- over the (S)-isomer, if the formed (S)- would eventually come in contact with the GIT mucosa.

Geisslinger *et al.* (1989) reported one of the first studies involving administration of a single 2-APA isomer in place of the usually administered racemate, to patients diagnosed with RA. After administration of either 500 mg b.i.d. or t.i.d. of (S)-ibuprofen for two weeks, none of the four patients studied experienced any gastro-intestinal distress or other side effects. These authors hypothesized the administration of the single (S)-enantiomer of ibuprofen to arthritic patients would result in a dose and metabolic load reduction. However, whether this would result in a lower incidence of side effects is only speculative due to the relatively short trial period. Until it is unequivocally shown that either the (R)-enantiomer contributes significantly to the side effects of the racemate and/or that the administration of the single (S)-enantiomer is more efficacious that the racemate, one must assume the racemate is the best dosage form.

As discussed in the preceding section, fifty RA patients underwent a study to compare the efficacy of (S)-ibuprofen (mean dosage: 1220 $\pm$ 60 mg/day) with that of the racemate (mean dosage: 1870 $\pm$ 140 mg/day). There was no statistically significant difference between the two groups in the number of patients who experienced gastro-intestinal pain and/or CNS disturbances (*i.e.* nausea and vomiting) (Stock *et al.* 1991).

In one of the few studies to report toxicity data, Buttinoni *et al.* (1983) reported the

acute LD_{50} toxicity of indoprofen in the rat. They found the (R)-enantiomer to have only 6% of the acute toxicity of its antipode. Because of this, the contribution of the (R)-isomer to the toxicity of the racemate would be expected to be minimal and therefore as expected, the toxicity of the (S)-isomer on a mg/kg basis was found to be twice the racemic mixture. It must be keep in mind that these were acute toxicity studies and chronic studies may yield different results. Furthermore, unexpectedly no significant difference in toxicity between oral and intravenous dosing was found.

It is well established (*vida supra*) that the 2-APAs have the ability *in vitro* to be substrates for the biosynthesis of hybrid triglycerides. Endogenous triacylglycerol biosynthesis occurs in adipose tissue and the liver as well as during absorption from the intestine. The fatty acids passively diffuse into the columnar epithelial cells of the intestinal mucosa. Within these cells, esterification of the fatty acids to fatty acyl CoAs and finally to triacylglycerols occurs. The re-esterification of triglycerides occurs in the citernae of the endoplasmic reticulum of the mucosal cells. In addition, the formation of chylomicrons and VLDLs are also synthesized in the mucosal cells. The formed chylomicrons containing the synthesized TGs are then released into the circulation via the lymphatic system. Before the TGs of the chylomicrons are taken up by tissues, they are hydrolysed to monoglycerols and FFAs by membrane bound lipoprotein lipase. Further hydrolysis of monoacylglycerols to glycerol and FFA occurs via a monoglycerol hydrolase (Orten and Neuhaus, 1982). As these locations are involved in endogenous TG biosynthesis, they can be considered potential areas for the biosynthesis of the xenobiotic triglycerides. Studies involving the aryl-containing xenobiotic 4-benzyloxybenzoic acid

(BRL 14280) with isolated rat liver, adipose and jejunal tissue were capable of forming hybrid triglycerides (Fears *et al.* 1978). It was subsequently shown that these xenobiotic triglycerides circulate in the lymph and plasma chylomicron and VLDL fractions, identical to normal processes (Fears and Richards, 1981).

Although there may theoretically be less GIT toxicity through the administration of the (R)-isomer, its administration could result in CNS toxicity above that normally observed with the racemate or assumed with the single (S)-enantiomer. It is conceivable that the 2-APAs could be deposited in neural tissue where an alteration of membrane function may occur with disturbances in lipid biochemistry (Caldwell 1984). CNS side effects of racemic flurbiprofen include headaches, dizziness, fatigue and insomnia (Brogden *et al.* 1979) while aseptic meningitis has been reported with ibuprofen (Giansiracusa *et al.* 1980)[10]. One example of a possible route of access to the CNS for the 2-APAs are as a thioester replacement for palmitoyl CoA-a precursor to a group of complex lipids called the sphingolipids, whose greatest concentration is in the white matter of the CNS. In addition, diglycerides can act as precursors to phospholipids such as phosphatidyl-ethanolamine, -choline and -serine of which 40% of brain tissue is composed. As mentioned previously, this would result from the ability of the (R)-enantiomer of certain 2-APAs to form hybrid triglycerides. However, although hybrid triglyceride uptake has been examined in adipose and hepatic tissue, to date there have

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[10]Aseptic meningitis has also been reported for tolmetin (Ruppert and Barth, 1981) which is achiral, and sulindac (Ballas and Donta, 1982), which although is a racemate, probably lacks the necessary structure and therefore has not been reported to undergo incorporation into hybrid triglycerides.

been no studies to determine whether they are deposited in the brain. Flurbiprofen has been shown to decrease prostaglandin levels in the brain of rats after oral administration (Brogden *et al.* 1979). Rats administered oral ^{14}C-flurbiprofen, a drug known to undergo little inversion in rats (Jamali *et al.* 1988b), found that the amount of radioactivity in the brain was only 1.53% of that found in the plasma over 24 hours (Ishii *et al.* 1975). This finding of low brain uptake is perhaps not unexpected, as the inability of FL to invert excludes it from hybrid formation. Hence, the observed CNS side effects of FL cannot therefore be due to hydrid formation. It is known however, that both ketoprofen (Netter *et al.* 1985) and pirprofen (Zecca *et al.* 1988) rapidly cross the blood brain barrier. Although the two agents exhibit considerably dissimilar CSF versus time profiles, both are detectable in the CSF within 30 minutes after a single intra-muscular injection. The CSF concentrations of pirprofen between 5 and 24 hours post-injection ranged 1-5% of plasma values. Although this would appear to be low, it is between 5 to 25% higher than the unbound fraction of calculated plasma pirprofen (Zecca *et al.* 1988). Unfortunately, neither of these two studies determined the individual enantiomer concentrations.

Hence, it appears the 2-APAs are detectable in the CSF and radioactive products are found in the brain. However, the relative enantiomer concentrations are not known nor whether the intermediate xenobiotic-CoA conjugates or the final hybrid triglycerides have significant biological effects. Does their formation constitute a slow metabolizing residual tissue store which ultimately may alter either cell membrane function and/or disrupt normal cellular metabolism (Caldwell, 1984)?

The role of these hybrid metabolites in producing toxicity or influencing therapeutic

activity by acting as a depot is uncertain.

1.4.3.3 Pharmacokinetic Considerations

When comparing racemate *vs.* the single enantiomer PK's, one must ensure the singly administered isomers attain similar blood (or tissue) levels as observed after racemate administration, *i.e.*, is there evidence of enantiomeric interaction?

Marketting the single (R)-enantiomer of those agents which undergo appreciable inversion will require considerable predictablity as to the extent of inversion-- such that the concentration-time profiles between the (R)- and (Rac)- formulations can be considered bio-equivalent. A similar argument can be made for the single (S)-enantiomer. The formulation is of primary importance when dealing with those 2-APAs which undergo inversion. Administration of a solution or rapid-release formulation may not allow sufficient contact time between the (R)-enantiomer and the GIT, thereby the degree and extent of inversion can be noticeably less. Even dosage forms commercially available from different manufacturers can give rise to significant differences in either the AUC as observed with flurbiprofen (Jamali *et al.* 1991b); T_{max} and C_{max} differences (Cox *et al.* 1988) or differences in the eudismic ratio as seen with ibuprofen (Jamali *et al.* 1988a; Borin *et al.* 1988).

1.4.3 Enantiomeric Inversion

The 2-APAs are unique among the NSAIDs: all members have the potential, although not necessarily the provision, to undergo a uni-directional *in vivo* enantiomeric

inversion, whereby the (R)-enantiomer or distomer may be inverted to the active antipode, the (S)-enantiomer or eutomer. The occurrence of the reverse reaction is controversial: with *in vivo* inversion in the rat of (S)-2-PPA suggested in one report (Fournel and Caldwell, 1986) and (S)-ibuprofen in the rat, guinea pig and rabbit by another group (Chen *et al.* 1990; 1991). This reverse reaction, however, has not been replicated *in vivo* by other workers or with other 2-APAs, and the possibility of antipode contamination cannot be discounted. Circumstantial *in vitro* evidence that (S)-FN and (S)-IB are capable of inversion is provided by Knadler and Hall (1990) and Saito *et al.* (1991). The former group, upon incubation of (R)-FN with (S)-FN impurity (approx. 3.5%) with rat liver homogenate, found CoA conjugates of (S)-FN. This was recently confirmed by Saito *et al.* (1991) using rabbit and rat microsomes incubated with coenzyme A and (S)-FN report the formation of a small amount of (S)-FN-CoA. Furthermore, Porubeck *et al.* (1991) using rat liver mitochondria and (R)- and (S)-flurbiprofen-CoA, have shown inversion of both enantiomers is possible. Accordingly, if (S)-thioesters are detected after addition of free acid as in the cases above, inversion appears possible. The extent or degree of (R)- to (S)- inversion is dependent upon drug, species and when examined *in vitro*, by the tissue, its method of preparation (*i.e.* whether isolated organ, tissue slice, isolated cell or homogenate) and the presence of obligatory co-factors.

The phenomena of enantiomeric inversion of the 2-APAs was first suspected with ibuprofen when it was found that regardless of the isomeric composition administered (*i.e.* individual enantiomers or racemate), the major urinary metabolites formed were

found to be (+)- or dextrorotatory (Adams *et al.* 1967), (now known to be the (S)-configuration) and later confirmed by Mills *et al.* (1973).

In man, inversion has been shown to occur with ibuprofen (Kaiser *et al.* 1976; Adams *et al.* 1976; Lee *et al.* 1985; Jamali *et al.* 1988a; Mehvar and Jamali, 1988), fenoprofen (Rubin *et al.* 1985) and benoxaprofen (Simmonds *et al.* 1980; Bopp *et al.* 1979), and as recently as 1988 was still considered a 'general one for the 2-arylpropionates' (Caldwell *et al.* 1988), a belief now known to be spurious. At least in man, it does not occur to any significant extent with tiaprofenic acid (Singh *et al.* 1986), indoprofen (Tamassia *et al.* 1984), carprofen (Iwakawa *et al.* 1987) and flurbiprofen (Jamali *et al.* 1988a).

1.4.3.1 Mechanism of Inversion

While it has been repeatedly demonstrated that the **process** of inversion occurs with selected 2-APAs (Kaiser *et al.* 1976; Lee *et al.* 1985; Rubin *et al.* 1985), several groups have attempted to discern the **mechanism** of inversion. Wechter *et al.* (1974) showed that the R-(-)- and not the S-(+)-enantiomer is a substrate for the 'isomerase' reaction and hypothesized the reaction proceeded *via* the enzyme system of lipid catabolism. Similar to long chain fatty acid catabolism, Wechter *et al.* (1974) suggested the initial reaction would involve the formation of a Co-enzyme A thioester, followed by reaction with fatty acid dehydrogenase losing the α-methine and one β-methyl (C-3) hydrogen to form a methylene, *i.e.* 2,3-dehydroibuprofen intermediate. They based their hypothesis on evidence collected using deuterium labelled ibuprofen and the subsequent partial

metabolic loss of that deuterium label. Also using deuterium substituted ibuprofen, the mechanism proposed by Nakamura *et al.* (1981) concurred with that suggested by Wechter *et al.* (1974) on the formation of a CoA thioester by loss of the α-methine proton. However, Nakamura *et al.* (1981) concluded that no β-methyl protons were lost, consistent against the formation of a methylene intermediate. These workers found that in both *in vivo* and *in vitro* preparations, only the (R)-enantiomer of susceptible 2-APAs, forms a CoA-thioester and therefore is the only isomer to undergo inversion. Interestingly, Nakamura *et al.* (1981) found that synthetic (S)-ibuprofen-SCoA will undergo epimerization to its antipode, clearly showing that CoA thioester formation is the determining step if subsequent inversion is to proceed. Implication of hepatic microsomal long chain acyl-CoA synthetase (E.C. 6.2.1.3) in the formation of the thioesters was confirmed using Wistar-strain rat liver microsomes for (R)-ibuprofen (Knihinicki *et al.* 1989) and (R)-fenoprofen (Knights *et al.* 1988).

Recently, Tracy and Hall (1991) have shown that upon incubation of the thioesters under conditions necessary to initiate hydrolysis (2N KOH heated at 55°C for 45 min.), both the (R) and (S)-CoA-thioesters may undergo a 16% epimerization to their respective antipode. However, it is not yet known which of the experimental conditions (*i.e.* the base and/or heat) causes the inversion. This question is important as some authors (Knihinicki *et al.* 1989) have used base without heat to carry out their hydrolysis of the thioesters. The epimerization is assumed to occur with the thioester, as the free enantiomers of the 2-APAs (e.g. flurbiprofen) are known to be stable in solution and under conditions of prolonged high temperatures (Szpunar, 1984).

In a revealing investigation previously mentioned, it has been shown that the inability of (R)-flurbiprofen to form thioesters is the reason for this enantiomers almost complete lack of inversion. Using a liver mitochondrial preparation, Porubek *et al.* (1991) administered synthetic (R)- and (S)-flurbiprofen-SCoA to a rat liver mitochondrial preparation and subsequently both enantiomers were shown to undergo almost complete epimerization. Therefore, as opposed to the suggestion of Mayer *et al.* (1989) that inversion may be chemically mediated, this study gave convincing evidence that inversion is indeed enzymatic. The ability of the 2-APA enzyme to carry out bidirectional inversion of the (R)- and (S)-arylpropionic acid enantiomers is not surprising and would be consistent with other mammalian and microbial racemases. Several racemase enzymes are known: alanine racemase (Wang and Walsh, 1978, 1981), methylmalonyl CoA racemase (Stabler *et al.* 1985) and mandelate racemase (Whitman *et al.* 1985), and all accept both enantiomers. Mandelate racemase, for example, binds the two enantiomers with equal affinity and carries out isomer inversion at equal rates of reaction (Whitman *et al.* 1985). Therefore, the observation that epimerization of (R)- and (S)-IB-CoA in rat and human liver preparations lacks stereospecificity with the exhibition of similar rates of reaction (Tracy and Hall, 1992), is in agreement with the above racemases. As mentioned earlier, enzymatic stereospecificity is an assumed fundamental premise due to the enzymatic composition of single isomer amino acids. As an active site is intrinsically asymmetric, an incongruity exists with the ability of racemase enzymes to catalyze both enantiomers. Using mandelate racemase as an example, it has been suggested that upon binding the individual enantiomers, a pseudo-symmetric environment

is established, with any differential binding of each enantiomer being compensated by side chain flexibility (Whitman *et al.* 1985).

Present information regarding the inversion pathway and related reactions is summarized in Figure 1.1.

In a series of papers, Baillie and co-workers (Baillie *et al.* 1989; Sanins *et al.* 1990; Sanins *et al.* 1991) studied the mechanism of ibuprofen in the human, rat and *in vitro* using isolated rat hepatocytes. After oral administration to humans, they confirmed earlier reports that there was no loss of protons from the β-methyl (C-3) position. These authors have suggested the conflicting observations reported by Wechter *et al.* (1974) discussed above, were probably the result of using incompletely labelled deuterium-[$^2H_3$]-ibuprofen as starting material.

The two major metabolic products of ibuprofen catabolism, hydroxyibuprofen (OH-IB) and carboxyibuprofen (COOH-IB) have long been identified (Mills *et al.* 1973), but surprisingly, with the exception of the parent (R)-isomer, research to date has yet to establish which metabolic species are capable of undergoing inversion. Figure 1.2 is a representation of all *possible* metabolic pathways that may occur after administration of (R)-ibuprofen. Referring to the figure, it is not known whether: (1) inversion occurs strictly with the administered (R)-parent forming (S)-IB (pathway B) and that all subsequent metabolites arise solely from the formed (S)-IB (Pathways B^2, B^3, B^4) and/or (2) inversion ensues with the susceptible metabolites (pathways A^2, C^2, D^2).

Therefore, three of the six ibuprofen metabolites may originate *via* more than one pathway. Knowledge of the contribution by each pathway to the formed metabolites is

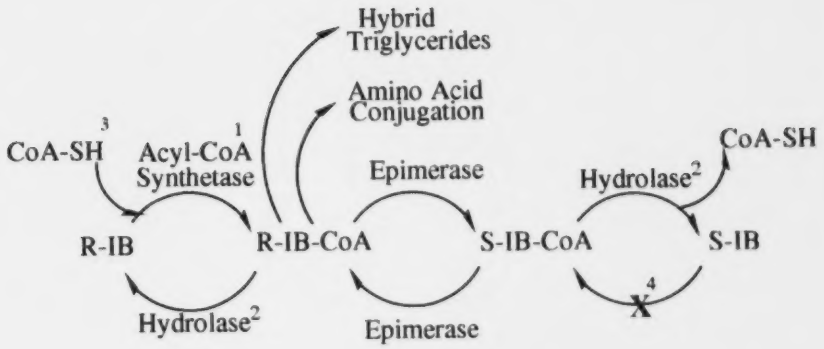


Figure 1.1 Proposed schema for the inversion of ibuprofen.

[1]The formation of the CoA-thioester apparently is the only process in the scheme which exhibits stereoselectivity. Epimerization and thioester hydrolysis are non-stereoselective for ibuprofen when examined in S-D rat and human liver preparations (Tracy and Hall, 1992).

[2]Hydrolase activity is apparently under co-factor (ATP and/or CoA) concentration-dependent feedback control whereby elevation in co-factor concentrations results in a reduction in the rate and extent of hydrolysis of the ibuprofen-CoA thioester (Tracy and Hall, 1991).

[3]Pantothenic acid, a substituent of co-enzyme A, is optically active making (R)-IB-SCoA and (S)-IB-SCoA diastereomers or epimers and with inversion only one of the two chiral centres is inverted by the 'epimerase' making the (R)-IB-SCoA to (S)-IB-SCoA reaction an epimerization. The overall reaction, however, of (R)-IB to (S)-IB can be termed a inversion as a single (and only) chiral centre of (R)-IB is inverted.

[4]Denotes reaction does not take place.

essential to accurately determine the extent of inversion of all species. Nevertheless, Baillie *et al.* (1989) calculated the percentage of inversion of ibuprofen and its metabolites with the assumption that any formed (S)-IB does not undergo further metabolism, *i.e.* reactions B^2 through B^4 did not occur. Therefore, these workers determined the extent of inversion of (R)-hydroxyibuprofen (R-OH-IB) as the ratio of (S)-hydroxyibuprofen (S-OH-IB) formed over the sum of (R)- and (S)-hydroxyibuprofen, with the assumption any (S)-OH-IB detected had as its sole precursor (R)-OH-IB.

However, as seen in Figure 1.2, there are two potential sources of (S)-hydroxy-ibuprofen: either from the aforementioned inversion of (R)-hydroxyibuprofen (pathway A^2) and/or from the further metabolism of the (S)-ibuprofen (pathway B^2) formed via inversion of administered (R)-ibuprofen. Therefore, the extent of inversion of (R)-hydroxyibuprofen to (S)-hydroxyibuprofen as calculated by Baillie *et al.* (1989) may be substantially underestimated. Furthermore, the extent of inversion of carboxyibuprofen may also be underestimated as there are two possible precursors of (SS)-carboxy-IB (pathways B^4 and D^2) and (SR)-carboxyibuprofen (pathways B^3 and C^2). Subsequently, the measured (SS)- and (SR)-carboxy-IB may not formed from (RS)- and (RR)-carboxy-IB respectively, but may instead be formed only from (S)-IB. In other words, perhaps **only** (R)-IB is capable of inversion. The metabolites (*i.e.* (R)-hydroxy-IB, (RS)- and (RR)-carboxy-IB) once formed, do not undergoing inversion and the presence of (S)-hydroxy-IB, (SS)- and (SR)-carboxy-IB have been formed solely from (S)-IB following inversion of the administered (R)-IB.

Assuming the chiral centre is not modified upon metabolite formation (which is a

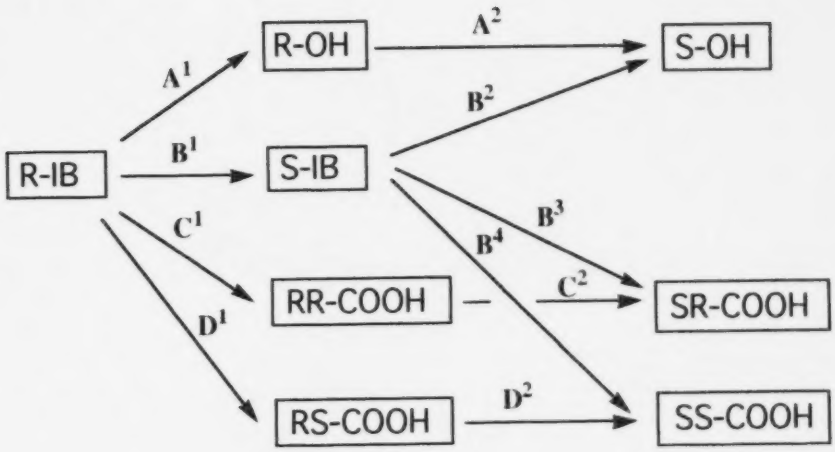


Figure 1.2 Potential pathways for ibuprofen enantiomer metabolism.

R-IB: R-ibuprofen; **S-IB**: S-ibuprofen; **R-OH**: R-hydroxyibuprofen; **S-OH**: S-hydroxyibuprofen; **RS-**, **RR-**, **SS-**, and **SR-COOH**: stereoisomers of carboxyibuprofen

reasonable assumption since phase I metabolism occurs distal to the chiral carbon), does inversion occur with (R)-hydroxy-IB, (RR)- and/or (RS)-carboxy-IB? Acceptance or rejection of the assumption will result in either an under- or over-estimation of the degree of inversion, respectively. In order to discern the pathway(s), administration of (RS)- or (RR)-[$^2H_1$] (C-2 methine proton) carboxy-IB, with either loss of label or formation of (SS)- or (SR)-carboxy-IB respectively, will confirm the occurrence of inversion; or administration of (R)-hydroxy-IB with subsequent examination for (S)-hydroxy-IB formation.

Therefore, it is clear that the relationship between the hydroxy- and carboxy-IB metabolites and inversion has not been well defined and more research is needed in this area.

Recently Rudy *et al.* (1991) administered (R)-, (S)- and Rac-ibuprofen to eight healthy volunteers and subsequently calculated the fractional inversion of (R)-ibuprofen. However, in doing so, these authors made similar assumptions as those made by Baillie *et al.* (1989): that the metabolites of (R)- and (S)-ibuprofen do not undergo inversion. As discussed above, until the possibility of inversion of selected metabolites is addressed, a correct determination of fractional inversion remains unresolved. In addition, Rudy *et al.* (1991) found a larger variability in clearance of (S)-ibuprofen after racemate administration relative to (R)-ibuprofen administration. The authors suggest this due to a time invariant clearance but although briefly discussed, failed to place enough importance on the possibility of enantiomeric interaction following administration of the racemate. These workers also suggest that discrepancies between studies may be the

result of the unknown disposition of approximately 30% of an administered dose. However, if all or part of this proportion is covalently bound in hybrid triglycerides, present methods will be unsuccessful in determining the ibuprofen disposition. Administration of suitably labelled (eg.^{11}C) ibuprofen to a suitable animal model with subsequent temporal whole body imaging should answer this question.

Chen and co-workers (Chen *et al.* 1990; 1991), also using deuterium labelled ibuprofen, further confirmed the loss of the single methine proton resulting in the formation of a carbanion intermediate, as the mechanism of inversion. The inversion is therefore due enhancement of the acidity of the methine proton (Caldwell, 1984) by the thioester formation and the aromatic ring. This facilitates the α-methine proton abstraction mediated by an acyl-CoA epimerase.

1.4.3.2 Tissue/Organ Location of Inversion

As with any orally administered drug, before reaching the systemic circulation it may undergo first-pass metabolism (*i.e.* phase I and/or II) with the gastro-intestinal tract and liver being the major organs involved in presystemic metabolism. Xenobiotic biotransformation by the GIT may occur via host enzymes present in the gut lumen and/or in the mucosal epithelial cell layer or by gut lumen microflora (Hartiala, 1973; George, 1981). The metabolic processes present in the GIT are similar to those found in the liver but generally of less activity.

The site(s) of inversion for the 2-APAs have been a subject of debate, with pre-systemic intestinal metabolism in man (Simmonds *et al.* 1980; Jamali, 1988; Jamali

et al. 1988) and hepatic systemic metabolism in rats (Cox *et al.* 1985; Muller *et al.* 1990a) receiving the most attention, although other organ involvement has been suggested (Yamaguchi and Nakamura, 1987; Knadler and Hall, 1990). As the investigation of site-specific metabolism in man has obvious difficulties, the use of isolated organs from animals can provide specific information.

Recently, there have been an increasing number of *in vitro* studies carried out on different systems to discern which organ(s) are responsible for inversion. These are summarized in Table 1.2 (*in vitro* hepatic inversion studies) and Table 1.3 (*in vitro* extrahepatic inversion studies).

Using tissue homogenates, a few reports have claimed that the liver and kidney are the major organs of inversion (Nakamura and Yamaguchi, 1987; Chen *et al.* 1991), and other organs such as intestinal tissue invert to a relatively small extent. However, one must view the results with caution, as one of these reports (Chen *et al.* 1991) added pre-synthesized ibuprofen-CoA thioester to Wistar rat tissue homogenates and were able to detect the antipode. This approach, although it may demonstrate the occurrence of inversion in a particular tissue, it may also provide misleading information, as entry to the site of inversion as well as thioester formation may both be important rate-limiting steps. Support for this argument originates from the work of Chen *et al.* (1991), who reported considerable inversion in Wistar rat kidney homogenates after addition of chemically synthesized (R)-ibuprofen-CoA. However, using the S-D rat IPK, Ahn *et al.* (1989 and 1991) perfused (R)-ibuprofen at different concentrations with subsequent analysis of both the perfusate and urine for ibuprofen isomers. Results indicated that

there was no evidence of inversion in the S-D rat kidney, however one cannot exclude the possibility of differences between Wistar and Sprague-Dawley species. Other reports have also indicated minimal inversion in the kidney (Knihinicki *et al.* 1989; Knadler and Hall, 1990).

Table 1.2 Summary of *in vitro* hepatic inversion studies[1]

Drug[2]	Animals[3]	Preparation	% inversion[6]	Reference
R-BN	Wistar rat	liver homogenate	0	Simmonds *et al.* (1980)
R-FL	S-D rat	liver mitochondria	0	Porubek *et al.* (1991)
R-FL	guinea pig[nr]	liver homogenate	0	Mayer *et al.* (1988)
Rac-KT	Wistar rat	liver homogenate	16	Lombard *et al.* (1985)
Rac-KT	Wistar rat	liver microsomes	21	Lombard *et al.* (1985)
R-IB	rat[nr]	liver homogenate	—[5]	Nakamura *et al.* (1985)
R-IB	rat[nr]	perfused liver	—[5]	Nakamura *et al.* (1985)
R-IB	S-D rat	perfused liver	—[5]	Cox *et al.* (1985)
R-IB	Wistar rat	liver homogenate	21[4]	Knihinicki *et al.* (1989)
R-IB	rat[nr]	liver homogenate	24	Knights and Addinall (1990)
R-IB	S-D rat	isolated hepatocytes	40[4]	Muller *et al.* (1990)
R-IB	S-D rat	isolated hepatocytes	11.5	Sanins *et al.* (1990)
R-IB	Wistar rat	liver homogenate	0	Chen *et al.* (1991)
Rac-IB	guinea-pig[nr]	liver homogenate	0	Mayer *et al.* (1988)

Table 1.2 Summary of *in vitro* hepatic inversion studies[1]

Drug[2]	Animals[3]	Preparation	% inversion[6]	Reference
R-NA	Wistar rat	liver homogenate	0	Mayer *et al.* (1988)
R-PPA	S-D rat	liver homogenate	0	Nakamura and Yamaguchi (1987)
R-PPA	S-D rat	liver slices	0	Nakamura and Yamaguchi (1987)
R-SU	guinea-pig[nr]	liver homogenate	0	Mayer *et al.* (1988)

[1] Only those studies which administered the free acids are included, *i.e.*, does not include studies where the CoA-thioesters were administered.

[2] **Rac-**: racemate; **R-**: R-enantiomer; **BN**: benoxaprofen; **KT**: ketoprofen; **FL**: flurbiprofen; **IB**: ibuprofen; **NA**: naproxen; **PPA**: 2-phenylpropionic acid (hydratropic acid); **SU**: suprofen.

[3] **S-D**: Sprague-Dawley; **nr**: strain not reported.

[4] Not reported--estimated from data.

[5] Inversion observed-extent not reported or calculation not possible

[6] Differences in calculation method may lead to discrepancies (*i.e.* calculated as percentage of initial starting R-isomer or as percentage of total (R + S) parent drug recovered when initial R-isomer quantity not reported).

Table 1.3 Summary of *in vitro* extrahepatic tissue inversion studies

Drug[1]	Animals[2]	Preparation	% inversion[5] @ incubation time	Reference
R-BN	Wistar rat	everted duodenum	17.4 @ 4h	Simmonds *et al.* (1980)
		caecum microflora	0	
		small intestine microflora	0	
R-FN	human	ileum[6]	0 @ 3h	Jamali *et al.* (1991)
		colon[6]	3.4 @ 3h	
	S-D[7] rat	small intest. microflora & luminal contents	2-5 @ 4h	Sattari and Jamali (1991)
		small intestine epithelial layer	28-35 @ 4h	
		small intestine muscular layer	3-6 @ 4h[11]	
		everted small intestine	26-32 @ 4h	
		germ-free[8] everted small intestine	28-33 @ 4h	
	Wistar rat	diaphragm homogenate	24.1[10]	Knadler and Hall (1990)
		lung homogenate	< 1[10]	
		kidney homogenate	1.8[10]	
		small intestine homogenate	< 1[10]	
		caecum homogenate	< 1[10]	
		skeletal muscle homogenate	3.2[10]	
		fat homogenate	3.2[10]	

Table 1.3 Summary of *in vitro* extrahepatic tissue inversion studies

Drug[1]	Animals[2]	Preparation	% inversion[5] @ incubation time	Reference
R-IB	human	ileum[6]	33.3 @ 3h	Jamali *et al.* (1991)
		colon[6]	34.5 @ 3h	
	Wistar rat	lung homogenate	< 1[10]	Knadler and Hall (1990)
		diaphragm homogenate	13.8[10]	
		small intestine homogenate	< 1[10]	
		caecum homogenate	< 1[10]	
		skeletal muscle homogenate	6.4[10]	
		kidney homogenate	1.2[10]	
		fat homogenate	< 1[10]	
R-IB-CoA	Wistar rat	lung homogenate	–[9]	Chen *et al.* (1991)
		heart homogenate	–[9]	
		kidney homogenate	–[9]	
		small intestine homogenate	–[9]	
		skeletal muscle homogenate	–[9]	
R-PPA	S-D rat	small intestine slices	3 @ 1h	Nakamura and Yamaguchi (1987)
		lung slices	0	
		testes slices	0	
		heart slices	0	
		spleen slices	0	

[1] **Rac**-: racemate; **R**-: R-enantiomer; **BN**: benoxaprofen; **KT**: ketoprofen;**FL**: flurbiprofen; **FN**: fenoprofen ; **IB**: ibuprofen; **NA**: naproxen; **PPA**: 2-phenylpropionic acid.

[2] **S-D**: Sprague-Dawley.

[3] Not reported and estimated from data.

[4] Inversion observed-extent not reported or calculation not possible.

[5] Differences in calculation method may lead to discrepancies (i.e. calculated as percentage of initial starting (R)-isomer or as percentage of total R + S parent drug recovered when initial R-isomer quantity not reported).

[6] Portion of tissue excised from three cancer patients--not known if portion used was cancerous and/or normal tissue.

[7] Female S-D rats.

[8] 500 mg/kg neomycin and erythromycin orally *b.i.d.* for 3 days prior to experiment.

[9] Expressed as recovered thioester.

[10] Percentage of total drug recovered as thioester after tissue incubation of R-isomers (i.e. not a measure of formed (S)-isomer as free acid).

[11] Observed inversion probably the result of epithelial cell contamination.

1.4.3.3 Cellular Location of Inversion

As discussed in a previous section (1.4.2.3), there is considerable evidence for the existence of either an intrinsic or analogous metabolic relationship between the chiral inversion of the 2-APAs and that of endogenous lipid metabolism. Therefore, a brief overview of the location(s) of cellular lipid metabolism is in order and the possible relationship to the location of inversion.

In lipid metabolism, fatty acyl-CoA formation in the cellular cytosol gains access to the mitochondrion by an initial exchange of the CoA moiety with carnitine and the subsequent transport of the fatty acid-carnitine complex into the mitochondrion whereupon the carnitine is exchanged for CoA from the mitochondrial pool. Therefore, it may be assumed the xenobiotic CoA intermediates have access to both the mitochondrial compartment as xenobiotic carnitine conjugates as well as the cytosol (Fears, 1985).

He *et al.* (1989) examined the ability of rat liver microsomes, mitochondria and cytosol to carry out conjugate formation between coenzyme A and (R)-fenoprofen. Liver microsomes had an extensive ability to carry out conjugation and inversion whereas the activity in the mitochondria and cytosol were low and negligible, respectively. In addition, these workers found human liver microsomes to have the ability to produce (R)-fenoprofen conjugates, but not ketoprofen conjugates.

Recently, Saito *et al.* (1991) using rabbit and rat microsomes, studied the *in vitro* coenzyme A conjugation with (S)-fenoprofen and the (R)-enantiomers of ketoprofen, carprofen and fenoprofen. Thioester formation was observed with (R)-KT and (R)-FN

while either little or no conjugates were formed with (S)-FN and (R)-CA respectively. These findings are consistent with that observed *in vivo*, reinforcing the belief that CoA thioester formation is necessary and the rate limiting step in the inversion process.

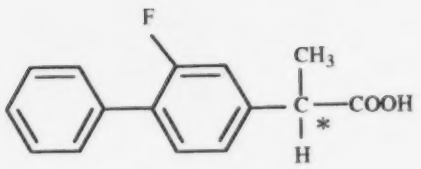
II. Rationale for Study and Research Objectives

2.1 Flurbiprofen

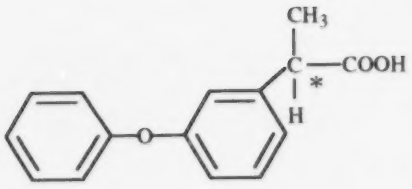
Flurbiprofen [FL, (±)-2-(2-fluoro-4-biphenylyl)propionic acid, Figure 2.1] is an anti-inflammatory/analgesic member of the 2-arylpropionic acid class. FL was originally developed by Boots Company (Nottingham, U.K.) and has been marketed in Europe since 1977 as an oral analgesic/anti-inflammatory under the trade-names *Froben®*, *Fluorofen®* or *Cebutid®* (Sunshine, 1986). In Canada, Organon Canada Ltd. (Mississauga, ON.), a Boots subsidiary, has marketed flurbiprofen as *Froben®* since 1986. Upjohn Canada Ltd. (Don Mills, ON.) also currently markets flurbiprofen in Canada (1986) and the U.S. (1988) under the name *Ansaid®*. In North America, oral flurbiprofen has been approved for use in the treatment of rheumatoid arthritis (RA) and osteoarthritis (OA) and acute gouty arthritis (Brooks *et al.* 1990). In addition, an ophthalmic preparation, flurbiprofen sodium is marketed as *Ocufen®* by Allergan for the inhibition of intraoperative miosis. To date, all clinically administered forms of flurbiprofen, whether for systemic or topical administration, are marketed as the racemate.

The pharmacology and metabolism of racemic flurbiprofen in several species including man have been reported (Glenn *et al.* 1973; Adams *et al.* 1975; Adams, 1977; Ridsall *et al.* 1978). Clinical efficacy and safety studies of flurbiprofen have been reviewed recently (Stubbs and Freeman, 1989; Brooks *et al.* 1990).

At the genesis of this project, a review of the literature revealed that a majority of the assays for FL were non-stereoselective, that is, they failed to differentiate between



Flurbiprofen



Fenoprofen

Figure 2.1 Structures of flurbiprofen and fenoprofen. Asterisk indicates chiral centre.

the two enantiomers. Only a few of the reported FL assays were stereospecific, but these suffered from a variety of drawbacks. For example, Maître *et al.* (1984) reported a general enantiospecific HPLC method for the 2-APAs, requiring a derivat-ization procedure which was both time consuming ($\approx$ 90 min.) and necessitated two clean-up extractions before injection. A stereospecific GC method was reported by Singh *et al.* (1986) which required long incubation times ($>$ 2 h) and an elevated temperature (85°C) for derivatization. Initially, therefore, the development of a rapid and sensitive assay was necessary. The assay must be capable of quantitating flurbiprofen enantiomers at concentrations normally encountered in plasma after recommended doses. This method, based on the derivatization procedure of Bjorkman (1985) with some modi-fications has previously been applied to the stereospecific assay of ketoprofen (Foster and Jamali, 1987) and fenoprofen (Mehvar and Jamali, 1988).

No doubt the consequence of the lack of a rapid and sensitive assay, there had not been a previous stereoselective pharmacokinetic study of FL conducted in man or animal. The next logical step after assay development was an investigation into the disposition kinetics of the individual enantiomers of flurbiprofen in man and a suitable animal model. In the course of that study, the single enantiomer (R)-FL was administered to a subject in order to determine whether inversion was possible in man. A serendipitous observation in that subject led to the hypothesis that in both man and rats, the two enantiomers of FL mutually interact when the racemate is administered. This hypothesis was examined using the Sprague-Dawley rat as a model, by administration of single IV doses to three groups of rats, each member of a group receiving a single IV dose of

either racemic, (R)- or (S)-FL.

2.2 Fenoprofen

In order to study the process of inversion, a drug and an animal model that can approximate or approach the observed pharmacokinetics in man is needed. Fenoprofen [FN, (±)-2-(3-phenoxyphenyl)propionic acid, Figure 2.1], like flurbiprofen, is also an anti-inflammatory/analgesic member of the 2-arylpropionic acid class and was originally developed by the Eli Lilly Company. FN has been previously demonstrated to rapidly undergo substantial *in vivo* uni-directional chiral inversion. Of the 2-APAs studied to date, FN undergoes the largest extent of inversion yet observed (Rubin *et al.* 1985).

As with any orally administered drug, before reaching the systemic circulation, FN may undergo first-pass metabolism (i.e. phase I and/or II) the major organs involved being the gastrointestinal tract and/or the liver. The site(s) of inversion for the 2-APAs have been a subject of debate, with pre-systemic intestinal metabolism in man and hepatic systemic metabolism in rats receiving the most attention. The inversion of (R)-FN was studied in the male Sprague-Dawley rat 1) *in vivo* after oral and intravenous administration of rac-FN and (R)-FN, 2) after perfusion of (R)-FN into isolated liver (single-pass and recirculation) and 3) following a 2 h incubation with everted intestinal tissue (duodenum, jejunum, ileum and colon) and non-everted stomach pouch. Enantiomers and acyl-glucuronide conjugates were quantitated using a previously developed stereospecific assay.

The most extensive inversion in man is observed with fenoprofen, while inversion

with other 2-APAs is not as complete. Xenobiotic biotransformation by the GIT may occur via host enzymes present in the gut lumen and/or in the mucosal epithelial cell layer or by gut lumen microflora (Hartiala, 1973; George, 1981). The metabolic processes present in the GIT are similar to those found in the liver but generally of less activity.

The site(s) of inversion for the 2-APAs have been a subject of debate, with pre-systemic intestinal metabolism in man and hepatic systemic metabolism in rats receiving the most attention (Jamali, 1988; Jamali *et al.* 1988a). As the investigation of site-specific metabolism in man has obvious difficulties, the use of isolated organs from animals can provide specific information. Therefore, the purpose of the present work is threefold: 1) to determine if the Sprague-Dawley rat is a suitable model for the study of inversion in man, 2) to discern whether fenoprofen inversion is presystemic and/or systemic, and 3) to identify organs involved in inversion.

III. Materials and Methods

3.1 Materials

HPLC-grade acetonitrile (UV cut-off 210 nm) was purchased from Caledon Laboratories Ltd. (Georgetown, ON.). Water (for extraction), potassium phosphate monobasic, triethylamine, and iso-octane were all HPLC-grade or reagent grade and purchased from Fisher Scientific (Ottawa, ON.). L-leucinamide was purchased from Sigma Chemical Co. (St. Louis, MO., USA). Chloroform was analytical-reagent grade supplied by BDH Chemicals (Toronto, ON.). Mobile phase water was glass distilled and filtered (Corning Mega-Pure SA-70 System, Corning, N.Y., USA).

Flurbiprofen racemate, individual R-(-) and S-(+) isomers were kindly supplied by Organon Canada Ltd. (Westhill, ON.). Internal standard (IS) ketoprofen was supplied by Rhone-Poulenc Pharma Inc. (Montreal, PQ.). Commercially available tablets of flurbiprofen (*Ansaid*® Upjohn: Toronto, ON.) were purchased from a local pharmacy. Purity of S- and R-enantiomers were 98.6 and 99.0%, respectively, as measured by a stereospecific assay (See Methods). Racemic and the (R)-enantiomer of fenoprofen were kindly supplied by Eli Lilly and Company (Toronto, ON.). Purity of the (R)-enantiomer was 98-99%, as measured in our laboratory using a previously reported stereospecific HPLC assay (Mehvar and Jamali, 1988).

Aspartate transaminase (ATA) and alanine aminotransferase (AAT) levels in perfused liver effluent were measured using a commercially available kit (Sigma Chemicals Inc., St. Louis, MO. USA).

3.2 Methods

3.2.1 Assay for Flurbiprofen Enantiomers

3.2.1.1 Standard Solutions and Calibration Curves

Racemic flurbiprofen, 10 mg, or fenoprofen, 10 mg, was dissolved in 20 mL methanol and made up to 100 mL with water (Solution A). A 10 mL aliquot of this solution was further diluted to 100 mL with 20% methanol (Solution B). Internal standard racemic ketoprofen, 10 mg (stereospecific method IS), or naproxen, 10 mg (non-stereospecific method IS), were dissolved in 20 mL methanol and made up to 100 mL with HPLC-grade water. All standards were extracted in the same manner as described below for plasma samples. To 0.5 mL of plasma or urine was added different aliquots of the above solutions to produce final concentrations of 0.1 to 20 mg/L (6 points) and 0.25 to 100 mg/L (9 points), respectively.

Calibration curves using standard solutions were prepared as follows:

Final Conc., mg/L	FL added for final 0.5 mL plasma volume
blank	0.5 mL drug-free plasma
0.1	10 μL of Solution B
0.5	50 μL of B
1.0	100 μL of B
5.0	50 μL of A
10.0	100 μL of A
20.0	200 μL of A

Calibration standards were prepared as follows:

Final Conc., mg/L	Vol. FL (10mg/100 mL)	Vol. blank plasma
blank	0 μL	150 μL
6.67	10 μL	140 μL
16.67	25 μL	125 μL
33.34	50 μL	100 μL
66.67	100 μL	50 μL

The standard samples were then treated in the same manner as described below for plasma and urine samples.

For the *in vitro* isolated tissue studies, fenoprofen was dissolved in 0.1N NaOH.

3.2.1.2 Extraction Procedure

To 0.5 mL of plasma was added 200 μL 0.6M H_2SO_4. To hydrolyse conjugated FL in urine, 100 μL of 1M NaOH preceded the acid, vortexing between additions. To this was added 50 μL of IS (100 mg/L) followed by 3 mL of extracting solvent, isopropanol:iso-octane (5:95 v/v). This mixture was mixed vigorously for 45 sec then centrifuged at 3000 rpm on a Clay-Adams centrifuge for 5 min.

The top layer was transferred to a clean tube and 3 mL HPLC-grade water was added. The mixture was again mixed vigorously and centrifuged for 5 min. The organic layer was aspirated off and 350 μL of 0.6M H_2SO_4 was added to the remaining aqueous layer.

This was mixed with 3 mL of chloroform and centrifuged for 5 min. The aqueous

layer was aspirated off and the remaining chloroform layer was evaporated to dryness using a SVC100H Savant Speed Vac Concentrator and Refrigerated Condensation Trap (Emerston Instruments, Scarborough, ON.).

Using the above method, the extraction efficiency was determined in urine and plasma samples spiked with Rac-flurbiprofen (1.0 mg/L and 5.0 mg/L , n=3) in the absence of IS. Following the final chloroform extraction and evaporation, the residue was dissolved in 200 μL of methanol with 50 μL of IS and the resultant solution was chromatographed by direct injection. The area ratios (Rac-FL/IS) were compared with the direct injection of a similar solution without extraction. For this purpose, the samples were analyzed by a non-stereospecific HPLC assay (Jamali *et al.* 1984 and 1985).

3.2.1.3 Derivatization Procedure

The derivatization scheme is represented in Figure 3.1. To the evaporated residue was added 100 μL of 50 mM triethylamine (TEA)(final concentration) in acetonitrile (AC), followed by, at 30 second intervals, 50 μL of 60 mM ethyl chloroformate in AC, then 50 μL of a 1 M L-leucinamide hydrochloride solution, prepared by dissolving the L-leucinamide initially in methanol (approx. 5% of final volume), then q.s. with TEA. After 2 min 50 μL of HPLC-grade H_2O was added and a 10-50 μL aliquot of this mixture was used for analysis.

3.2.1.4 Chromatographic Procedure

A Waters Associates HPLC system (Rexdale, ON.) consisting of a Model 6000A

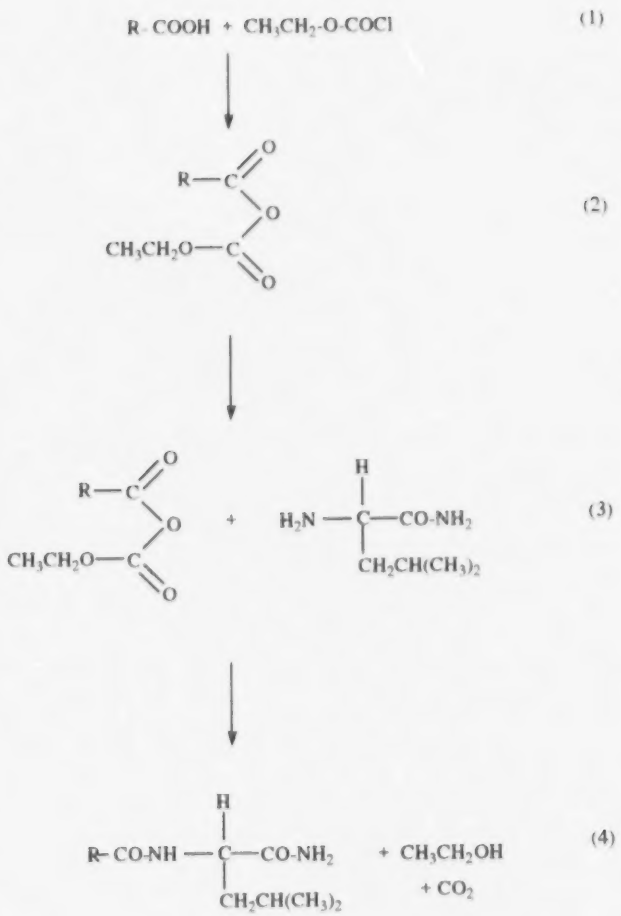


Figure 3.1 Derivatization scheme for the quantitation of flurbiprofen enantiomers.

(1) FL (R-COOH) derivatized with ethyl chloroformate forming a mixed anhydride (2) which further reacts with an amine, L-leucinamide (3) forming the final amide diastereomer (4).

pump, 710B WISP auto-injector and a Model 490 Multiple-wavelength UV detector was operated at ambient temperature. Detection of IS was carried out at 275 nm while flurbiprofen was detected at 250 nm. The chromatographic column was stainless steel (100 mm x 4.6 mm i.d.) packed with 5-μm size octadecyl-bonded silica (Partisil 5 ODS-3, Whatman Inc. Clifton, NJ., USA) preceded by a 5-cm guard column containing 10-μm particle size octadecyl-bonded silica. The area ratio of flurbiprofen over IS was used for all quantitation using a Hewlett-Packard 3390A integrator-recorder (Hewlett-Packard, Palo Alto, CA., USA). The filtered (0.45 micron) mobile phase consisted of acetonitrile:0.067M KH_2PO_4: triethylamine (35:65:0.02 v/v) pumped at a flow rate of 1.0 mL/min. at ambient temperature.

3.2.1.5 Accuracy and Precision

The intra- (n=4) and inter- (n=6) day variations of the assay were examined for solutions containing 0.1, 1, 10 and 20 mg/L.

3.2.2 Non-Stereospecific Assay for Flurbiprofen

To determine extraction efficiency, the non-stereospecific assay used was based on that of Jamali *et al.* (1984 and 1985) briefly described below:

To a 1.5 mL micro-centrifuge tube (Fisher Scientific) was added 150 μL sample plasma or standard plasma, 50 μL of IS (naproxen, 10 mg/100 mL) and 300 μL HPLC-grade acetonitrile. The sample was vortexed for five seconds and then centrifuged at high speed (Fisher micro-centrifuge, Model 235A) for 10 minutes. The samples were

then directly injected (10-50 μL) into the chromatograph.

All apparatus was as described for the stereospecific assay of flurbiprofen with the following exceptions: Model 491 fixed-wavelength UV detector with IS (naproxen) and flurbiprofen detected at 254 nm. The mobile phase consisted of acetonitrile: 0.067M KH_2PO_4:triethylamine (32:68:0.02 v/v).

3.2.3 Assay for Fenoprofen Enantiomers

Quantitation of the enantiomers of fenoprofen used the methods of Mehvar and Jamali (1988). The method involved extraction of the drug from the biological specimen with isopropanol/isooctane (5/95% v/v), back extraction into water and then chloroform, evaporation of the organic layer and addition of 1) TEA/AC 2) ethyl chloroformate/AC and 3) L-leucinamide/methanol:TEA. After addition of water, the solution was injected into an HPLC equipped with a UV detector at 232 nm for fenoprofen and 275 nm for internal standard, ketoprofen). The mobile phase consisted of acetonitrile: 0.067M KH_2PO_4:triethylamine (35:65:0.02 v/v) pumped at a flow of 1 mL/min through a stainless steel (100 mm x 4.6 mm i.d.) end-capped column packed with 5-μm size octadecyl-bonded silica (Partisil 5 ODS-3, Whatman Inc. Clifton, NJ., USA) preceded by a 5-cm guard column containing 10-μm particle size octadecyl-bonded silica.

3.2.4 Ethics Approval

All animal experimentation protocols were reviewed and approved by the University of Alberta Animal Care Ethics Committee.

All human studies were approved by the Human Ethic Review Committee of the University of Alberta Hospitals and each subject signed a written consent form.

3.2.5 Animals

All animals were of the Sprague-Dawley strain of *Rattus norvegicus* (Lindsey, 1979) raised in-house by the University of Alberta Animal Colony Facility.

3.2.6 Rat Studies: *In Vivo*

3.2.6.1 Jugular Vein and Biliary Duct Cannulation

The male Sprague-Dawley rats were anaesthetized initially with ether and then maintained on methoxyflurane (*Metofane®*, Pitman-Moore LTD, Mississauga, ON.) during the procedure. The jugular vein and bile duct were cannulated with polyethylene tubing (PE-50, Clay-Adams, Parsipanny, NJ., USA) tipped with Silastic Tubing (Dow Corning) and Microbore Icon tubing respectively. Animals recovered overnight before drug administration.

3.2.6.2 Drug Administration and Sample Collection

For either IV or oral administration, flurbiprofen or fenoprofen were dissolved in polyethylene glycol (PEG) 400 at a concentration of 10 mg/mL racemate or 5 mg/mL for the single enantiomers. The solutions were injected (10 mg racemate/kg or 5 mg pure enantiomer/kg) into the jugular vein cannula which was then flushed with 0.1 mL of heparin (100 IU/mL) solution.

Blood samples (0.25 mL) were drawn from the jugular vein cannula at 0, 0.25, 0.5, 1.0, 2.0, 4.0, 6.0 and 8 h, and 12 h. Fenoprofen plasma samples were collected up to 24h. Bile was collected at 0, 0-2, 2-4, 4-6, and 6-8 h intervals. The specimens were centrifuged immediately on a Fisher Model 235A microcentrifuge for 3 minutes. All plasma, urine and bile samples were stored at -20°C until assayed.

3.2.6.3 Stereoselective Pharmacokinetics of Flurbiprofen Enantiomers

Eight male Sprague-Dawley rats (300-400 g) underwent jugular vein (n=8) and bile duct (n=2) cannulation as described in section 3.2.6.1. Sample collection and handling was as described in section 3.2.6.2.

Racemic flurbiprofen was administered to six rats, two of which had cannulated bile ducts. Due to the limited amount of available pure (R)-flurbiprofen only two additional rats were dosed with this enantiomer to examine the possibility of the enantiomeric inversion.

3.2.6.4 Enantiomeric Interaction of Flurbiprofen

Eighteen male Sprague-Dawley rats (300-450 g) were divided into three groups of six. The first group received the single enantiomer (R)-FL, the second group the single enantiomer (S)-FL with the last group receiving the racemate. All groups were administered their respective drugs intravenously. The rats underwent jugular vein cannulation as described in section 3.2.6.1. Flurbiprofen racemate and single enantiomers were administered and the samples collected as described in section 3.2.6.2.

3.2.6.5 Enantiomeric Dose-Range Study of Flurbiprofen

Male Sprague-Dawley rats (n=4-5/group; 210-295 g) were divided into six groups with each group to receive one of the following racemic flurbiprofen doses: 1.25, 2.5, 5.0, 10.0, 20.0, 40.0 mg/kg. After jugular vein cannulation (section 3.2.6.1), all groups received their respective dose intravenously with samples collected as previously described (section 3.2.6.2).

3.2.6.6 Stereoselective Pharmacokinetics of Fenoprofen

Male Sprague-Dawley rats (n=4/group; 200-250 g) were administered either (R)-, or Rac-FN by the IV or oral routes. The rats underwent jugular vein cannulation as described in section 3.2.6.1. Fenoprofen racemate and the single (R)-enantiomer were administered and the samples collected as described in section 3.2.6.2.

3.2.7 Human Studies

3.2.7.1 Stereoselective Pharmacokinetics of Flurbiprofen Enantiomers

Six healthy subjects, 5 males and 1 female, 19 to 40 years old and within 10% of normal body weight, provided informed written consent to participate. Subjects were fasted for at least 8 hours before and two hours after drug administration. Single 100 mg tablets of flurbiprofen were administered orally with 250 mL water. Blood samples were taken at 0, 0.25, 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0, and 12.0 h., by venipuncture, in NaF/K oxalate containing Vacutainer® tubes. Samples were centrifuged immediately and the separated plasma stored at -20°C until assayed. Urine was collected at 0-2, 2-4, 4-6,

6-8, 8-12 and 12-24 h.

In an exploratory study, a healthy man was dosed orally first with 25 mg of (R)-flurbiprofen and one week later with 50 mg (Rac)-flurbiprofen. Both doses were administered in gelatin capsules. Plasma and urine samples were collected as described above.

3.2.8 Rat Studies: *In Vitro*

3.2.8.1 Isolated Rat Liver Perfusion

The procedures used were essentially similar to those reported by Miller (1973) with minor modifications. Using male Sprague-Dawley rats, the liver was removed after cannulation of the portal vein, inferior and thoracic vena cava. In both, the non-recirculating (single-pass) and recirculating experiments, the liver was perfused in an anterograde manner with oxygenated (95:5 oxygen:carbon dioxide) cell-free Krebs-Henseleit bicarbonate buffer containing 2 g/L glucose, maintained at 37°C. To maintain an adequate oxygen supply, the perfusion rate was maintained at 3-4 mL/min/g liver. The liver was monitored by measurement of aspartate transaminase (ATA) and alanine aminotransferase (AAT) levels, the hydrostatic pressure and its general physical appearance. The ATA and AAT levels and hydrostatic pressure were constant throughout the experiment, which lasted less than 2 hours from removal of the liver.

In the single-pass method (n=4), twenty minutes after the removal and catheterization of the liver, (R)-FN (250 mg/L) was infused at two different rates via a Harvard infusion pump (Model 600) into the Krebs-Henseleit buffer inflow, in order to

achieve two different inlet concentrations. On three occasions the order of dosing had an initial inlet concentration of approximately 7 mg/L maintained for 10 to 20 minutes, then drug infusion stopped. After approximately 15 minutes the drug infusion was resumed at one-half the original rate, giving an initial inlet concentration of approximately 3.5 mg/L. On one occasion, the order of dosing was reversed (*i.e.* from low to high inlet concentration) to determine if order of administration altered kinetic parameters. The duration of each liver infusion was 45 to 90 minutes. Samples were continually taken from the inlet and outlet. All samples were frozen at -20°C until analysis. To determine the presence of ester glucuronides, effluent samples were divided into two groups, with 0.25 mL 1M NaOH added to one group before extraction. In the recirculating mode (n=2), from time 0 the liver was continually perfused from an oxygenated Krebs-Henseleit buffer reservoir containing 5 mg/L (R)-FN.

3.2.8.2 Everted Rat Intestinal Preparation

The procedure used was similar to that of Esposito and Csaky (1974) with slight modifications. Male Sprague-Dawley rats (n=4) were anaesthetized with ether and the intestines were removed through a vertical midline incision. All tissue segments were approximately 10 cm. After the bile duct was tied off, the duodenal segment was removed immediately distal to the pyloric sphincter. The jejunum was removed starting 30 cm distal from the pyloric sphincter, the ileum was immediately proximal to the caecum and the colon immediately distal to the caecum. The excised intestines were immediately placed in ice-cold (4°C) Krebs-Henseleit bicarbonate buffer (pH 7.4)

containing 2 g/L of dextrose. The intestines were then flushed with ice-cold buffer and everted on a glass rod. One end was tied off and the wet tissue weighed. The sac was then filled with drug-free, warmed (37°C) oxygenated (95:5 oxygen:carbon dioxide) buffer, placed in a warmed jacketed glass organ bath containing 50 mL of 5 mg/L R-FN in continually oxygenated Krebs-Henseleit bicarbonate buffer and incubated for 2 h. In addition, (R)-FN (5 mg/L) was incubated in a closed stomach pouch (non-everted) in a manner similar to the intestinal segments described above. After 2 h, the mucosal and serosal fluids were sampled and frozen at -20°C until analysis. To examine for the presence of ester glucuronides, samples were divided into two groups, with 0.25 mL 1M NaOH added to one group before extraction.

3.2.9 Data Analysis

The t½ of the terminal elimination phase of the plasma concentration-time curves was estimated using a regression equation. The area under the plasma concentration-time curve ($AUC_{0-\infty}$) was calculated by the trapezoidal rule for 0-12 h and for 12 h-∞ by dividing the plasma concentration at 12 hours by the terminal elimination rate constant (β) with the latter area never exceeding 15% of total. Systemic (Cl_{TB}) and oral ($Cl_{TB/F}$) clearances were calculated as dose/AUC(0-∞) and the apparent volume of distribution as $Vd/F = CL/\beta$, where F is the extent of absorption.

For the fenoprofen experiments, on the assumption that (S)-FN exhibits linear kinetics and that no enantiomeric interactions occur, the fraction of (R)-FN inverted *in vivo* (f_i) was calculated as:

$$f_i = \frac{AUC_S^R}{(AUC_S^{Rac} - AUC_S^R)}$$

where AUC_S^R and AUC_S^{Rac} are the area under the curve for (S)-FN after administration of (R)-FN and (Rac)-FN, respectively. This is based upon the assumption the AUC of (S)-FN alone is equal to the difference between the mean AUC of (S)-FN after (Rac)-FN administration and the mean AUC of (S)-FN after (R)-FN administration. Based on these calculations, the estimated dose of (S)-FN introduced was used to calculate clearance and the volume of distribution of (S)-FN. Clearance (Cl_{TB}) was therefore calculated as (estimated dose)/AUC. The volume of distribution at steady-state was calculated after IV doses as:

$$Vdss = MRT * Cl_{TB}$$

where MRT is the mean residence time.

With the isolated perfused liver, the hepatic extraction ratio was calculated from the expression:

$$ER = \frac{(AUC_{aff} - AUC_{eff})}{AUC_{aff}}$$

The hepatic clearance was calculated from the expression:

$$CL_{organ} = Q * ER$$

where Q is the rate of the inflow perfusate. The rate constants for elimination during the drug infusions were calculated from the slopes of the concentration (steady-state

concentration minus concentration at sampling times) *vs.* time plots by the method of residuals (Gibaldi and Perrier, 1984). The rate constants for elimination after the drug infusions were calculated from the slope of the drug concentration *vs.* time curves.

With the everted intestinal tissue preparations, amounts of enantiomer recovered are expressed as per g of wet pre-incubation tissue weight.

Statistical significance of the observed differences between concentrations as well as between the pharmacokinetic indices were examined using the paired or unpaired Student's t-test, one-way analysis of variance or the multiple comparison test of Scheffe, as deemed appropriate at $\alpha = 0.05$.

IV. Results and Discussion

4.1 Assay for Flurbiprofen Enantiomers

Representative chromatograms of drug-free plasma, plasma spiked with 1 mg/L racemic flurbiprofen and a healthy subject's plasma sample 12 h after 100 mg oral dose are depicted in Fig. 4.1. The retention times for the derivatized individual isomers of (R)- and (S)- flurbiprofen are 17 and 21 min respectively. Retention times of the derivatized isomers of (R) and (S) IS was 8 and 10 min respectively. Pure individual enantiomers were derivatized and chromatographed to determine the relative order of elution. There were no interfering peaks from the normal components of plasma.

In urine, FL appeared almost completely as readily hydrolysable conjugates (Fig. 4.2). In addition to the peaks representing hydrolysed conjugated enantiomers of FL, two additional major peaks were noted which are presumed to be enantiomers of a metabolite.

Excellent linear relationships ($r^2 > 0.996$) were found between the peak area ratios and concentrations. Typical regression lines through the data points could be described for both enantiomers in plasma and urine, respectively (Table 4.1).

The extraction efficiency was determined in spiked samples of human plasma containing IS comparing the peak-area ratios of FL (1.0 μg/mL and 5.0 μg/mL, n=3) before and after extraction. The samples were then analyzed by a non-stereospecific HPLC assay as outlined above. The extraction efficiencies of (R) and (S) enantiomers of flurbiprofen in plasma and urine were greater than 90% at both concentrations studied.

The efficiency of the derivatization determined by measurement of the peak

representing underivatized FL was approximately 100%.

Linearity was assessed by calculating the correlation coefficient for the concentration range of 0.1 - 20 μg/mL in plasma (six points) (Table 4.1). The correlation coefficient (r^2) was at least 0.996.

The intra- and inter-day variations (*i.e.* precision) of the assay ranged from 1.1 to 8.9% (Tables 4.2 and 4.3).

The limit of reliable quantitation was set at 0.1 μg/mL and 0.25 μg/mL for each enantiomer in plasma and urine, respectively, which encompasses the concentrations observed in a healthy volunteer (Fig. 4.3).

The assay was found suitable for the determination of flurbiprofen enantiomers in human plasma and urine following administration of recommended doses.

Table 4.1 Calibration Curve Data of (R)-and (S)-Flurbiprofen in Plasma and Urine

Flurbiprofen Isomer	Regression Equation[a,b]	R^2
Plasma 0.1-20 μg/mL		
(R)-isomer	Y=.708X - .018	0.998
(S)-isomer	Y=.705X - .015	0.998
Urine 0.25-100 μg/mL		
(R)-isomer	Y=.713X - .034	0.996
(S)-isomer	Y=.708X - .031	0.996

[a]X = flurbiprofen concentration; Y = peak area ratio of flurbiprofen to internal standard (ketoprofen).
[b]mean of five values

Table 4.2 Inter-Day Variability of Flurbiprofen Analysis in Plasma

Concentration μg/mL	Isomer	Mean Concentration μg/mL	Coefficient of Variation %
0.1	(R)	0.109	8.85
	(S)	0.102	8.95
0.5	(R)	0.514	4.26
	(S)	0.498	4.41
1.0	(R)	0.999	2.76
	(S)	0.994	2.55
5.0	(R)	4.584	2.33
	(S)	4.645	2.32
10.0	(R)	9.788	1.88
	(S)	9.846	2.06
20.0	(R)	20.227	1.67
	(S)	20.127	1.70

Table 4.3 Intra-Day Variability of Flurbiprofen Analysis in Plasma			
Concentration μg/mL	Isomer	Mean Concentration μg/mL	Coefficient of Variation %
0.1	(R)	0.103	3.24
	(S)	0.104	3.21
1.0	(R)	1.009	1.90
	(S)	1.004	1.89
10.0	(R)	9.926	1.26
	(S)	9.928	1.36
20.0	(R)	20.019	1.15
	(S)	20.013	1.20

Fig. 4.1 Chromatograms of drug-free plasma (A), plasma spiked with 1 mg/L FL (B), and a healthy subject's plasma sample at 12 h after 100 mg po dose (C). Key: (R)- (1) and (S)- (2) ketoprofen, and (R)- (3) and (S)- (4) flurbiprofen.

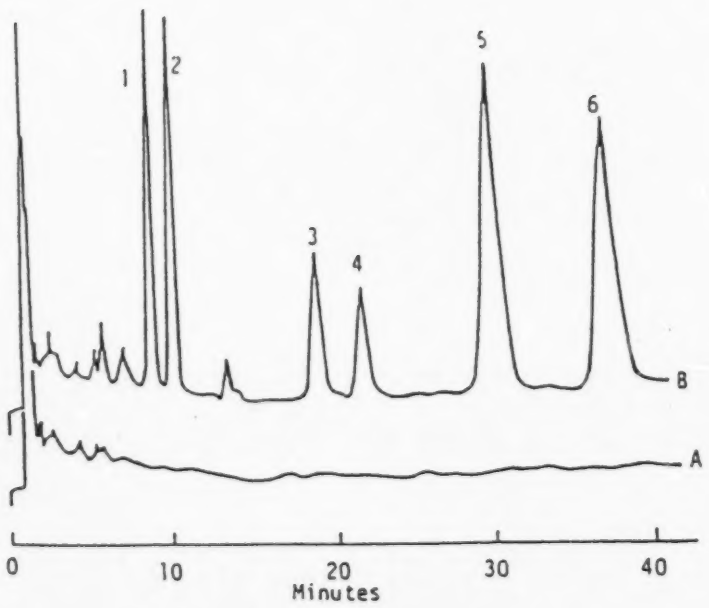


Fig. 4.2 Chromatograms of drug-free urine (A) and a healthy subject's 12-24 h urine sample after 100 mg dose (B) of racemic flurbiprofen. Key: 1, (R) and 2, (S)-ketoprofen; 3, (R)- and 4, (S)-flurbiprofen; 5 and 6 presumed enantiomers of a metabolite.

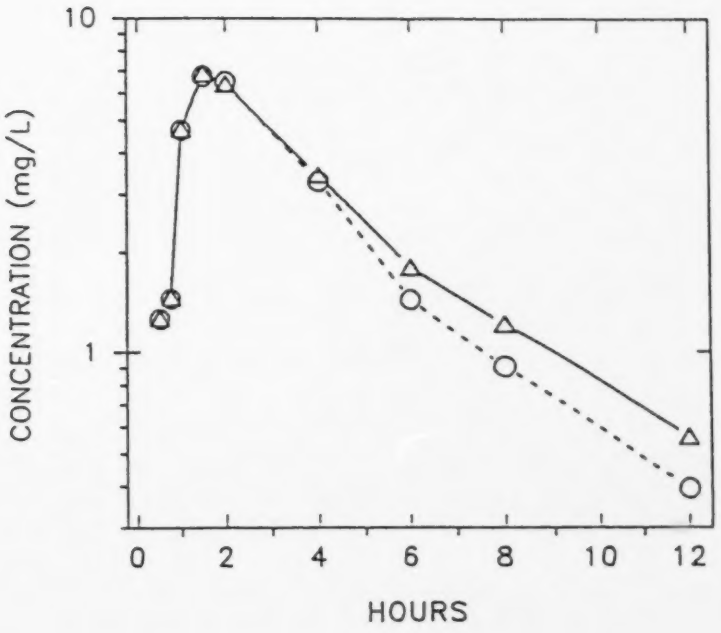


Fig. 4.3 Flurbiprofen enantiomer plasma concentrations versus time profile for a single healthy subject after 100 mg oral dose of racemic drug. Key: (S:△ ;R:○).

4.2 Stereoselective Pharmacokinetics of Flurbiprofen Enantiomers in Human and Rats

Typical time courses of flurbiprofen enantiomers in a rat as a representative of the sample population are depicted in Figure 4.4. The disposition kinetics of flurbiprofen in this species were stereoselective. In plasma, following racemic FL, (S)-flurbiprofen predominated [AUC, 134.14 ± 39.48 *vs.* 41.00 ± 8.86 (mg/L)h]. The (S)-enantiomer had a smaller Vd (67.1 ± 3.7 *vs.* 154.1 ± 33.4 mL) and longer t½ (4.14 ± 2.03 *vs.* 2.95 ± 0.92 h). The higher concentration of the (S)-flurbiprofen in the rat cannot be attributed entirely to an enantiomeric inversion because only a small concentration of that enantiomer was found in plasma after administration of (R)-flurbiprofen [AUC(R):AUC(S) = 0.10 and 0.16 for rats 1 and 2, respectively] (Fig. 4.5).

For carprofen the observed 2-fold difference in the clearance of (R)-enantiomer in the rat is attributed to its faster biliary excretion (Kemmerer *et al.* 1979). For flurbiprofen in the rat, however, the biliary route constitutes only a minor pathway of elimination. In total, only 5.2 and 3.6% of the administered racemic dose was recovered in bile as conjugated flurbiprofen, with S:R ratios being 2.1 and 1.2 for rats 1 and 2, respectively (Fig. 4.6). Only negligible amounts of unchanged flurbiprofen were detected in bile. The bile also contained a pair of unknown peaks, presumably, representing enantiomers of a metabolite of flurbiprofen. The areas of these peaks were approximately 50% of those of conjugated flurbiprofen with S:R ratios ranging from 1.5 to 4.6. Bile was collected for a period of approximately 2-3 t½s. The recovered amount, therefore, should constitute most of the total conjugated flurbiprofen expected to be excreted in bile.

In the bile duct cannulated rats, the plasma time-courses of the enantiomers of

flurbiprofen were similar to those in intact animals, suggesting little or no enterohepatic recirculation (Fig 4.6). This is consistent with the small quantity of the conjugated flurbiprofen enantiomers recovered in the bile. The possibility of enterohepatic cycling of flurbiprofen has previously been suggested (Risdall *et al.* 1978; Szpunar *et al.* 1987). However, in those studies ^{14}C-flurbiprofen was administered to the rats and total biliary radioactivity was measured, therefore, one cannot assess the contribution of individual excreted components.

Similar to the rat, the disposition of flurbiprofen in man is also stereoselective (Fig. 4.3, Table 4.4). In all patients except one (subject 6) the (S)-enantiomer concentrations were significantly higher in plasma, but the differences were not as prominent as in the rat. While there were no significant differences in t½'s and Vd's between enantiomers, the observed AUC's were smaller and CL's were greater for (R)-flurbiprofen to a significant extent.

Following IV doses of single (R)-flurbiprofen to the rat, only the administered enantiomer (with administered impurity) was detectable in plasma (Fig. 4.5) and urine. The above mentioned stereoselective disposition, therefore, cannot be attributed to enantiomeric inversion.

Similar to other 2-APAs (Tamassia *et al.* 1984; Upton *et al.* 1980; Jamali *et al.* 1985) only negligible amounts of intact flurbiprofen were found in urine. The urine contained mainly readily alkaline hydrolysable conjugates. They were conjugated enantiomers of flurbiprofen and of an unidentified metabolite, presumably 4'-hydroxy flurbiprofen, a metabolite which is reported to constitute 40 to 47% of the administered dose (Risdall

et al. 1978; Szpunar *et al.* 1987). The retention time of these peaks were identical to those of the unknown peaks observed in the rat bile. For both conjugated flurbiprofen and the metabolite, the (R)-enantiomer was predominant in urine. The R:S ratios for the metabolite varied from 1.6 to 1.0. These may indicate stereoselective hydroxylation and conjugation and also preferential urinary excretion of R-flurbiprofen.

An interesting observation made in both species is that following administration of single (R)-flurbiprofen the observed AUCs were considerably greater than those following the dose of racemate (Figs. 4.4, 4.5 and 4.8). This may represent an interaction between the enantiomers such as replacement from the plasma protein binding sites of (R)- with (S)-flurbiprofen followed by an enhanced clearance of the former. Stereoselective protein binding has been observed for other propionic acid derivatives (Rendic *et al.* 1980; Jones *et al.* 1986). Due to the limited amounts of available pure (R)-flurbiprofen and lack of sufficient assay sensitivity, a more detailed study was not possible to explore this hypothesis. Until this concern is addressed, the pharmacokinetics of the individual enantiomers following doses of the racemate cannot be extrapolated to those following administration of the pure enantiomer.

Fears *et al.* (1978) have shown that, in the rat, the 2-arylpropionic acid derivatives may replace one of the fatty acid moieties in the natural triglycerides and form unusual glycerolipids. The incorporation into the adipose tissue is suggested to occur during the R to S inversion process when (R)-coenzyme A thioesters are formed (Williams *et al.* 1986). The (S)-enantiomer, therefore, is expected to be excluded from this process rendering the uptake stereoselective. Consequently, the extent of the uptake by the

adipose tissue may be dependent upon the degree of the enantiomeric inversion. Interestingly, in the rat, ketoprofen with 80% R to S inversion (Foster and Jamali 1988) is found in the adipose tissue to a considerably greater amount than is flurbiprofen (Fears *et al.* 1978) with only a limited extent of inversion (Fig. 4.5).

Our observations present yet another example of the complex pharmacokinetics of chiral drugs that are administered as racemate. Interestingly, the reported pharmacokinetic patterns of not even two of these chemically related chiral NSAIDs are similar (Jamali, 1988). Similarly, the metabolic biotransformation of one enantiomer to the other observed for some of these compounds (Lee *et al.* 1985; Jamali *et al.* 1988; Mehvar and Jamali, 1988; Rubin *et al.* 1985; Simmonds *et al.* 1980) cannot be extrapolated to others (Singh *et al.* 1986; Tamassia *et al.* 1984; Iwakawa *et al.* 1987; Foster *et al.* 1988a and 1988b). Thus pharmacokinetic studies using stereospecific assays are appropriate for each individual member of this class of drug. Indeed, the differential pharmacologic activity of the (R)- and (S)- enantiomers makes such studies even more relevant, not only among NSAIDs, but also among other therapeutic agents administered as racemate.

Table 4.4 Pharmacokinetic Parameters of Flurbiprofen Enantiomers in Man After Oral Administration of a Single 100 mg Racemic Dose.

Subject[a]		1	2	3	4	5	6	Mean	SD
C_{max}, mg/L	R	8.2	13.4	9.8	5.9	4.7	6.6	8.1	3.2
	S	8.4	13.3	10.3	6.6	5.0	5.7	8.2	3.2
T_{max}, mg/L	R	1.5	0.75	0.75	1.0	2.0	4.0	1.67	1.2
	S	1.5	0.75	0.75	1.0	2.0	4.0	1.67	1.2
$t_{½}$, h^{-1}	R	3.04	4.82	2.53	4.00	4.76	5.90	4.18	1.3
	S	3.67	5.50	2.50	3.70	4.40	5.50	4.21	1.2
AUC_{0-12}, (mg/L)h	R	29.9	55.0	33.1	34.5	27.0	61.1	40.1	14.3
	S	35.5	66.6	40.8	40.3	34.3	54.7	45.4	12.7[b]
Cl_{TB}/F, L/h	R	1.67	0.91	1.51	2.06	1.85	0.82	1.47	0.50
	S	1.41	0.75	1.23	1.61	1.46	0.91	1.23	0.34[b]
V_d/F, L	R	7.31	6.32	5.53	11.63	12.7	16.98	8.41	3.0
	S	7.47	5.95	4.43	8.95	9.36	7.22	7.23	1.9
A_e, mg	R	9.89	11.17	10.58	9.67	11.12	5.9	9.72	2.0
	S	7.07	8.75	8.01	7.88	8.96	5.6	7.71	1.2[b]

[a] All male except subject 1

[b] Significantly different from R-flurbiprofen.

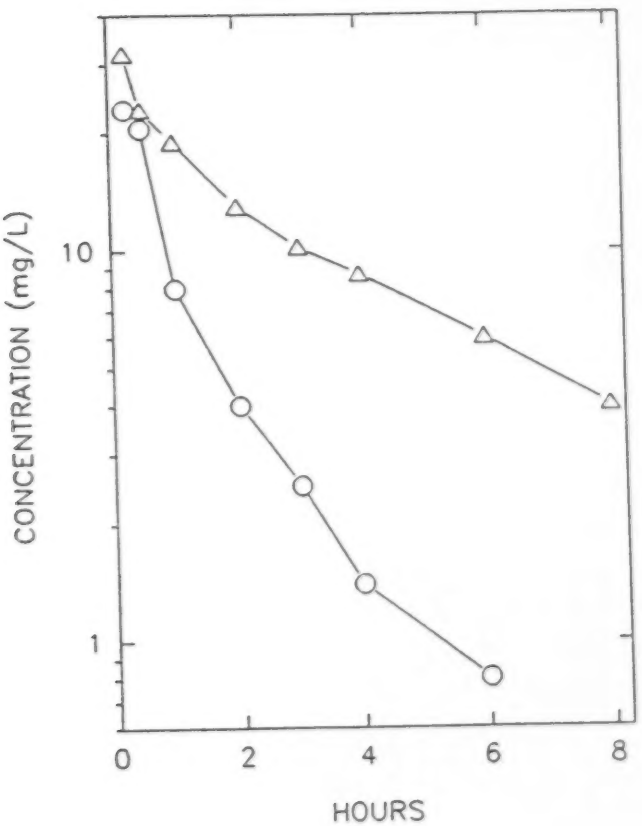


Figure 4.4 Plasma concentration *vs.* time-courses of flurbiprofen enantiomers in a rat following a 10 mg/kg IV racemic dose. Key:(S:△ ;R:○).

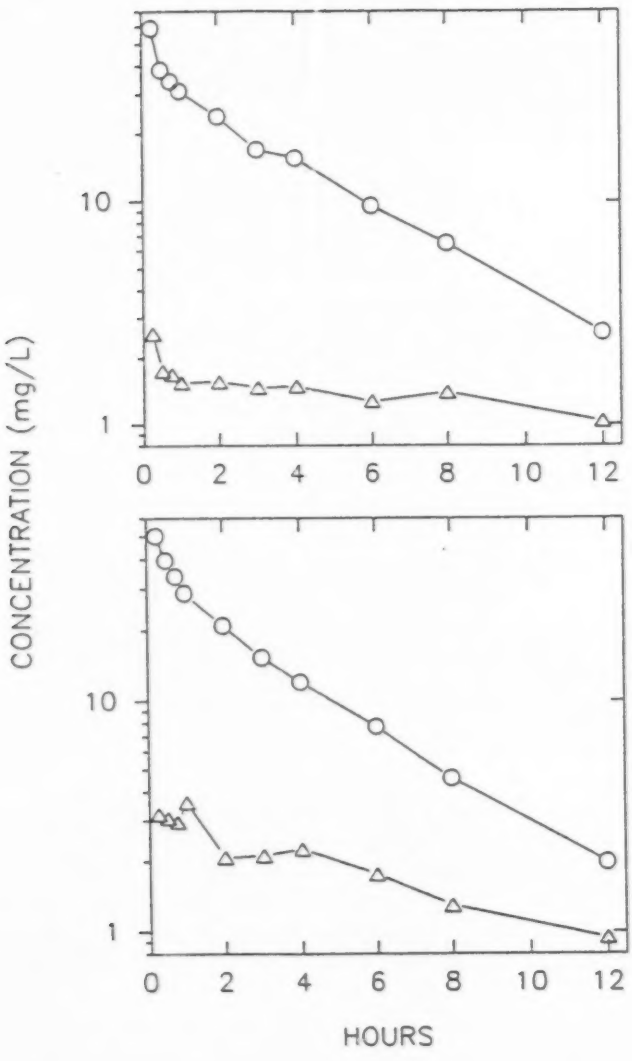


Figure 4.5 Plasma concentration *vs.* time-courses of flurbiprofen enantiomers in two rats following a 5 mg/kg IV dose of (R)-enantiomer. Key: (S:△ ;R:○).

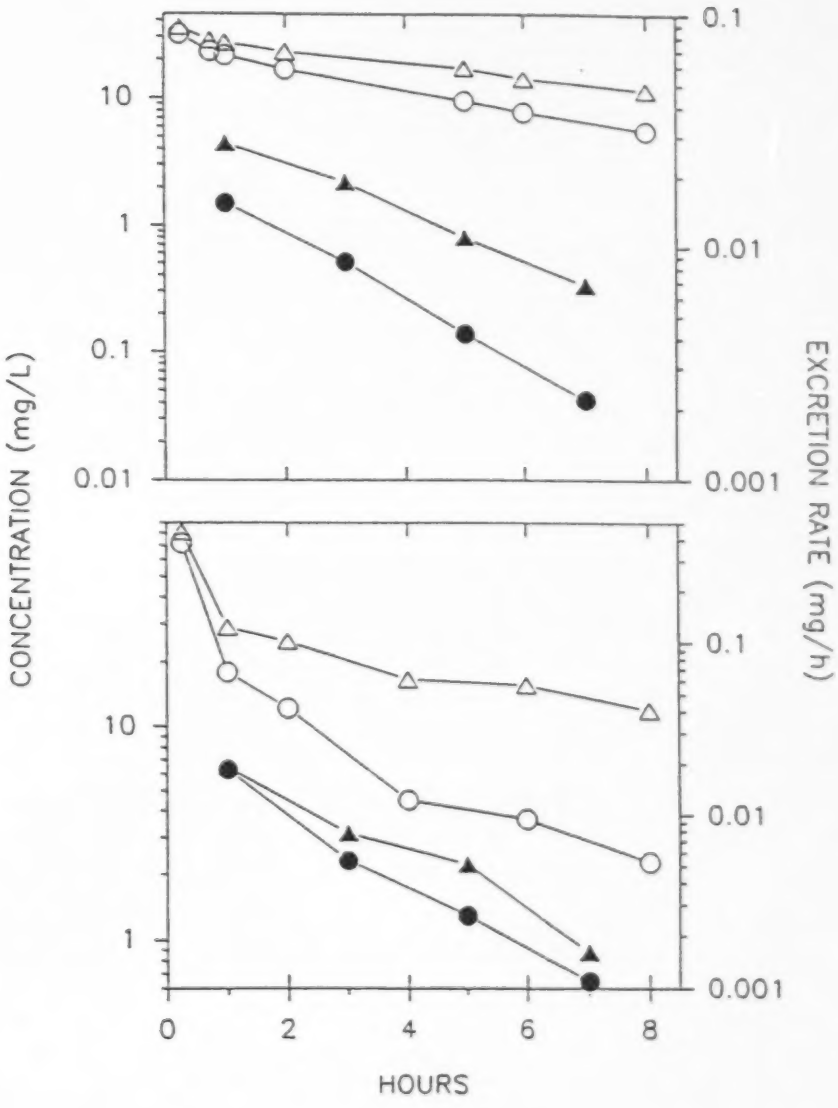


Figure 4.6 Plasma concentrations and biliary excretion rates of the flurbiprofen (S)- and (R)-enantiomers in two rats following a 10 mg/kg IV racemic dose. Key: Plasma-(S: ▵ ;R: ○); Biliary-(S: ▲ ; R: ●).

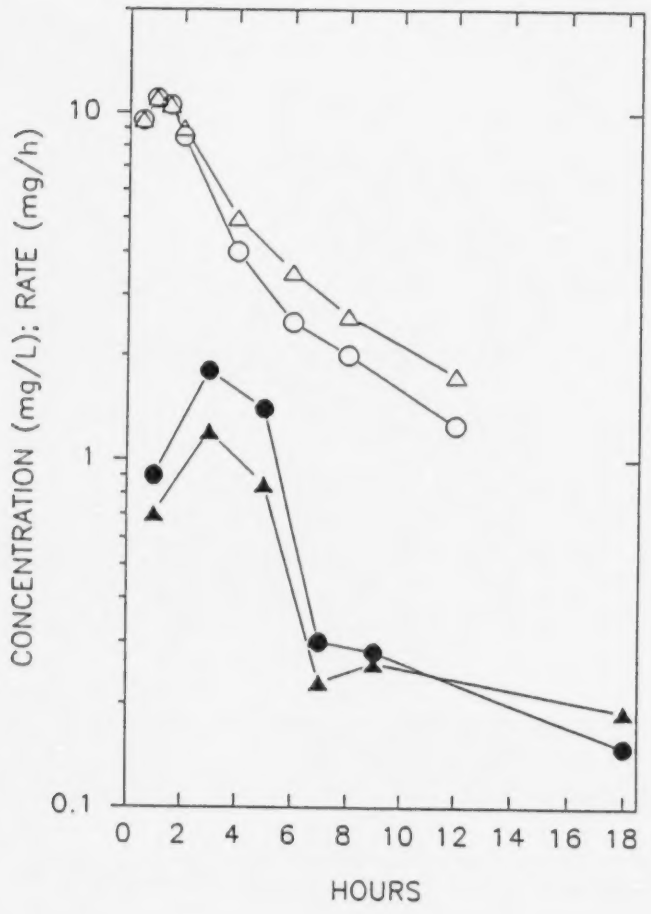


Fig. 4.7 Plasma concentrations and urinary excretion rates of flurbiprofen enantiomers in a healthy subject following a 100 mg oral racemic dose. Key: Plasma-(S:△ ;R:○). Urine-(S:▲ ;R:●).

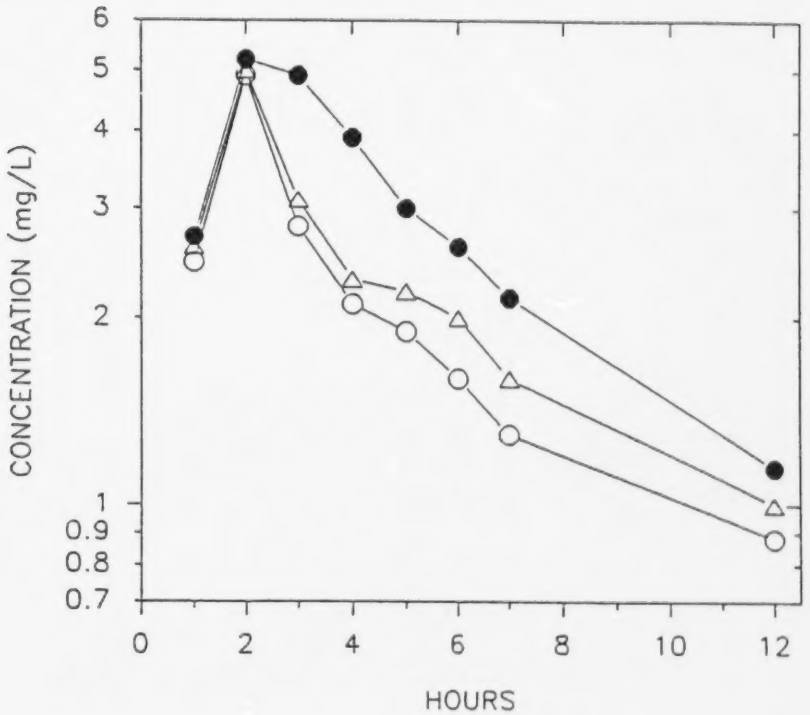


Figure 4.8 Plasma concentration *vs.* time-courses of flurbiprofen enantiomers in a healthy male subject following oral administration of a 50 mg racemic dose and a 25 mg dose of single (R)-enantiomer. Key: Racemate-(S:▵ ;R:○), Single R-(●).

4.3 Enantiomeric Interaction of Flurbiprofen Enantiomers

As shown in Table 4.5 and Fig. 4.9, following administration of racemic FL plasma concentrations of (S)-FL were significantly greater than those of (R)-FL (mean AUC^S/AUC^R = 3.60). This was coupled with a significantly longer terminal t½ for (S)-FL (4.55 ± 0.95 h *vs.* 3.06 ± 0.80 h). These findings are in agreement with our previous observation in the rat and man (Jamali *et al.* 1988b). The observed stereoselectivity in the pharmacokinetic pattern of the two enantiomers may be explained, in part, by enantiomeric inversion of (R)- to (S)-FL (Jamali *et al.* 1988b). Unequivocal proof of inversion, however, can only be found if after administration of one enantiomer the other is detected. Accordingly, when (R)-FL was administered alone, a significant plasma concentration of its antipode (over that attributed to enantiomeric impurity) was observed (mean AUC^S/AUC^R = 0.15) (Table 4.5, Fig. 4.9). After administration of (S)-FL alone, (R)-FL was observed in plasma (mean AUC^R/AUC^S= 0.022). However, the amount of (R)-FL present after (S)-FL administration can be attributed to cross enantiomeric impurity of the administered dose.

To calculate the extent of (R)- to (S)- inversion, (S)- alone must be administered and its AUC compared with those derived from (R)- after either (R)- alone or a dose of racemate. This, however, holds valid only when the enantiomer exhibits linear kinetics and in the absence of an enantiomer-enantiomer interaction. For FL these conditions were not met: a statistically significant *in vivo* interaction was observed between FL enantiomers, with the AUC of (R)-FL being 115.3 ± 21.3 (mg/L)h when given alone and 48.4 ± 10.4 (mg/L)h when administered as part of a racemic dose. A similar trend

Table 4.5 Pharmacokinetic Indices after Intravenous Administration of Racemic, S-, and R-Flurbiprofen to the Rat

	AUC, (mg/L)h		$t_{½}$, h		CL,mL/min/kg		V_{dss}, mL/kg	
	R	S	R	S	R	S	R	S
Racemate	51.9	204.1	3.30	5.26	1.60	----	319	----
10 mg/kg	50.2	174.2	2.32	2.95	1.70	----	274	----
	33.8	107.1	1.65	3.50	2.90	----	363	----
	39.3	167.4	3.56	5.26	2.51	----	478	----
	52.8	177.9	3.67	5.15	1.84	----	369	----
	66.2	229.8	3.85	5.15	1.26	----	338	----
Mean	49.0	176.8[c]	3.06	4.55[c]	1.97	----	357	----
SD	10.4	37.7	0.80	0.95	0.56	----	62	----
R-FL,	109.5	14.6	2.58	---[a]	0.77	----	176	----
5 mg/kg	75.6	15.8	2.80	6.60	1.12	----	267	----
	115.9	10.6	2.10	2.42	0.70	----	159	----
	115.2	20.7	2.22	3.48	0.71	----	148	----
	131.4	11.1	3.86	---[a]	0.92	----	210	----
	144.4	29.6	3.03	6.30	0.58	----	140	----
Mean	115.3[b]	17.1	2.77	4.70	0.80[b]	----	183[b]	----
SD	21.3	6.5	0.58	1.79	0.17	----	44	----
S-FL,	5.40	115.9	9.76	4.03	----	0.72	----	243
5 mg/kg	3.80	164.1	---[a]	3.79	----	0.49	----	185
	10.60	361.4	10.04	9.35	----	0.23	----	182
	5.20	322.9	7.79	4.85	----	0.27	----	117
	2.70	211.2	---[a]	5.16	----	0.39	----	181
	4.60	271.4	4.52	6.13	----	0.31	----	151
Mean	5.38	241.2	8.03	5.55	----	0.40	----	177
SD	2.50	86.2	2.20	1.86	----	0.17	----	38

[a]Not calculated due to excessive fluctuation in plasma concentration. [b]Significantly different from racemate. [c]Significantly different from the coadministtred R-FL.

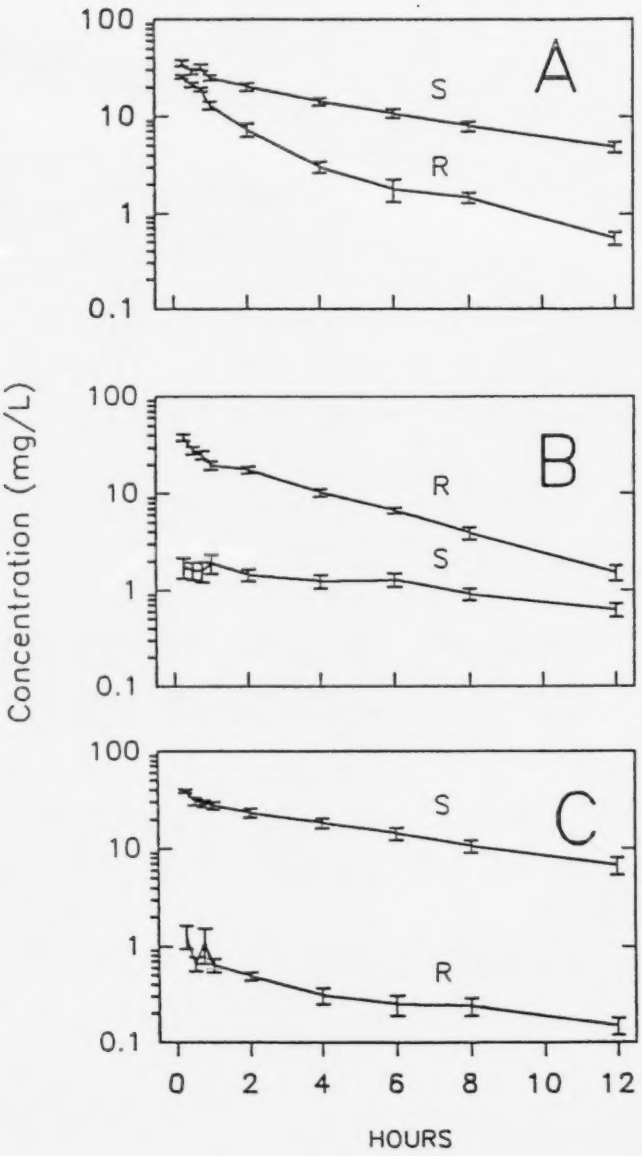


Figure 4.9 Mean plasma time courses of flurbiprofen enantiomers in the rat following intravenous administration of **(A)** 10 mg/kg racemate and 5 mg/kg each of the **(B)** (R)- and **(C)** (S)-enantiomer. Key: Enantiomers as labelled. The presence of (R)-FL after (S)-FL administration (C) represents enantiomer impurity. Bars are standard errors of the means.

was also present for (S)-FL (AUC: singly: 241.2 ± 86.2 *vs.* racemate: 176.8 ± 37.7). This decrease, however, was not statistically significant. Accordingly, for (R)-FL when given singly, values for Cl_{TB}: (0.24 ± 0.09 *vs.* 0.60 ± 0.12 mL/min) and Vd were decreased (57.2 ± 22.0 *vs.* 151.6 ± 26.2 mL) to a statistically significant level while t½ remained unaffected as compared to those after racemic doses. Due to the observed interaction, the fraction of the administered (R)-FL inverted to (S)-FL, and consequently Cl_{TB} and Vd of (S)-FL cannot be calculated with any degree of confidence.

It appears that the pharmacologically active (S)-enantiomer enhances clearance of its antipode. A trend towards the reverse (*i.e.*, increased clearance of S by R) also exists. Such an interaction may take place at various levels such as competition between the enantiomers for binding to plasma proteins and/or induction of metabolism.

The 2-APAs are not known to possess enzyme inducing or inhibiting properties. Although FL is suggested to reduce clearance of antipyrine by inhibition of hepatic enzymes (Chalmers *et al.* 1973), it does not seem to alter its own metabolism after chronic administration (Cardoe *et al.* 1975; 1977). Alteration of metabolism, therefore, is an unlikely mechanism for the observed interaction. A change in the extent of protein binding, on the other hand, is expected to significantly alter the clearance of this highly bound, slowly cleared drug (Wilkinson and Shand, 1975). Hence, the substantial increase in Cl_{TB} and V_{dss} of (R)-FL that resulted from co-administration of (S)-FL may suggest an interaction at the level of protein binding. This postulate is in line with the observation of Knadler *et al.* (1988). Using human plasma, these authors have shown *in vitro* stereo-selective protein binding for FL whereby both enantiomers appear to compete for a

common binding site. They showed the (S)-enantiomer has increased avidity toward the plasma proteins over that of the (R)-enantiomer, particularly at concentrations greater than 26 mg/L. At this concentration range, significant displacements of one enantiomer by the other were also observed (Knadler *et al.* 1988). Unfortunately, we were unable to study the protein binding of FL enantiomers due to the lack of sufficient sensitivity of our assay.

Competitive enantiomer-enantiomer protein binding has also been suggested for other 2-APA drugs (Jamali *et al.* 1989a). The parent congener of this group, 2-phenylpropionic acid (PPA), although not used clinically, has been studied extensively as a model compound for this class. In the rat, Yamaguchi and Nakamura (1985) found a slightly higher plasma protein binding of (R)-PPA over that of the S enantiomer in both *in vivo* and *in vitro* studies. Using rabbit plasma, Jones *et al.* (1986) showed that enantiomeric competition occurs between the enantiomers of 2-PPA with each enantiomer competitively lowering the protein binding of the other. Lee *et al.* (1985), studying the disposition of ibuprofen in humans, showed a statistically significant increase in AUC of the (R)-ibuprofen given alone as compared to racemate. The authors suggested an enantiomer-enantiomer interaction leading to a perturbation in protein binding to be responsible for their observation. At low concentrations of ketoprofen, a preferential binding of the R enantiomer to human serum albumin has been shown, but the possibility of enantiomeric interaction was not studied (Rendic *et al.* 1980). Nevertheless, this group did show that stereoselective binding can be influenced by protein concentration. It is interesting to note that the observed enantiomeric interaction is apparently not a

universal process for the 2-APAs as Tamassia *et al.* (1984) did not observe a significant difference between the AUCs of the (S)-enantiomer of indoprofen administered singly or as part of a racemate.

A reduced number of plasma protein binding sites resulting from the proposed competition of the enantiomers increases the unbound fraction of the drug and enhances elimination and/or other tissue uptake. Saturation of protein sites is believed to be the underlying mechanism for nonlinearity in pharmacokinetics of drugs such as naproxen (Runkel *et al.* 1974) and ibuprofen (Lockwood *et al.* 1983). Such a mechanism may also explain the observed several fold larger Vd of (+)-etodolac as compared to its antipode after adminstration of a racemic dose to man (Jamali *et al.* 1988c).

Similarity of man and rat in disposing of FL (Jamali *et al.* 1988b) may make this observation therapeutically significant. This may be particularly important during active rheumatic diseases when the circulating plasma proteins are reduced (Baum *et al.* 1985; Van Den Ouweland *et al.* 1986)

In conclusion, several of the 2-APA members may display stereoselective protein binding, and the binding of one enantiomer may be altered by the other. The presence of an enantiomeric interaction renders questionable (Jamali *et al.* 1989a) the estimation of the extent of the R to S inversion, as carried out by some investigators (Lee *et al.* 1985).

4.4 Dose Study of Flurbiprofen Enantiomers in the Rat

In the previous study using the Sprague-Dawley rat, following administration of 10 mg/kg of racemic FL, an enantiomer-enantiomer interaction was observed. As is the case with all NSAIDs administered clinically to a diverse patient population, use of a variable dose range is common. With typically achieved therapeutic plasma concentrations, a few of the 2-APAs, notably ibuprofen (Lockwood *et al.* 1983) and naproxen (Runkel *et al.* 1974), have been shown to exhibit non-linear pharmacokinetics. The observed deviation from linearity has been attributed to a saturation of plasma protein binding sites. In addition, FL has been shown to exhibit non-linear protein binding in the therapeutic plasma concentration range (Szpunar *et al.* 1989). The authors of the latter study however, concluded that this minimal non-linearity has a less profound effect on the pharmacokinetics of FL than with ibuprofen.

In all the above mentioned studies, quantitation of the respective 2-APAs has been made as total drug rather than as individual enantiomers, using non-stereospecific assays. To date, there have been no reported enantiomeric interaction studies expressly carried out to examine the possible dose-related effects. Therefore, using the Sprague-Dawley rat as a model, racemic FL was administered at six different dosages to determine whether the previously observed enantiomeric interaction is dose-related.

Furthermore, the mechanism of enantiomeric interaction is proposed to involve competition of the antipodes at the level of protein binding. If so, the relative plasma concentrations of the enantiomers are expected to be dose-dependent, i.e. the higher the dose of the (S)-enantiomer, the more rapid becomes the clearance of the (R)-isomer.

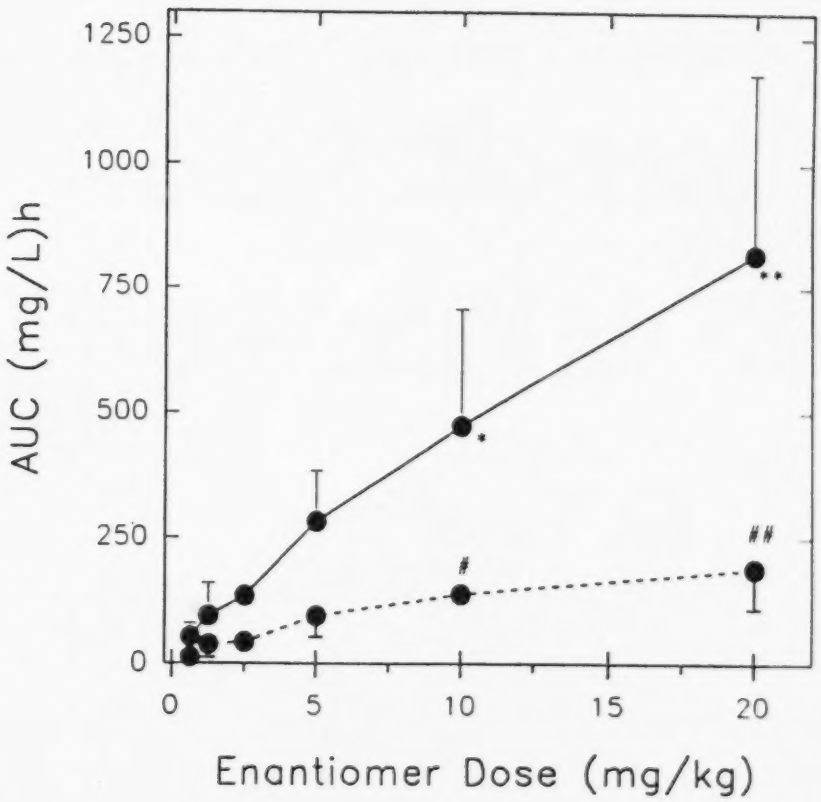


Figure 4.10 $AUC_{0-\infty}$ of the (R)- and (S)-flurbiprofen enantiomers *vs.* enantiomer dose following IV administration of six different **racemate** doses to the rat.

Key: Solid line-(S); Dotted line-(R). Symbols: *-Significantly different from point 1; **-Significantly different from points 1, 2, 3 and 4; #-Significantly different from points 1, 2 and 3; ##-Significantly different from points 1, 2, 3 and 4. Significance $p < 0.05$, Scheffe's Multiple Comparison Test.

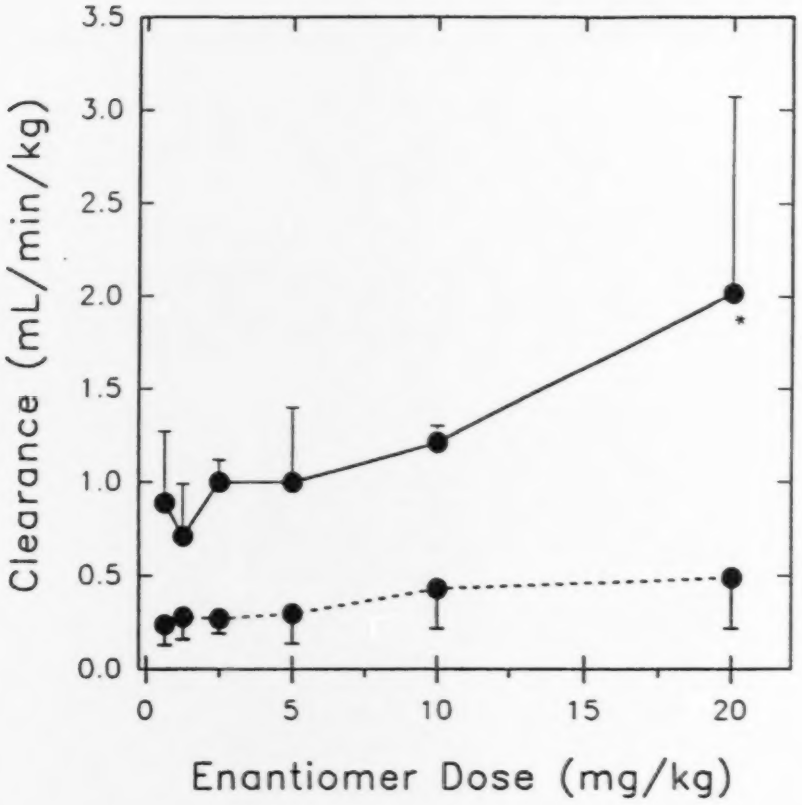


Figure 4.11 Clearance of the (R)- and (S)-flurbiprofen enantiomers *vs.* enantiomer dose after IV administration of six different **racemate** doses to the rat.

Key: Solid line-(R); Dotted line-(S). Symbols: *-Significantly different from point 1.

Table 4.6 Dose-Range Study of Flurbiprofen Enantiomers after Racemate Administration in the Rat[1]

Rac Dose, mg/kg	t1/2,h		AUC, (mg/L)hr		CL, mL/min/kg		Vdss, mL/kg	
	R	S	R	S	R	S	R	S
1.25	2.96 (0.90)	5.80 (2.96)	14.11 (7.35)	52.39 (28.07)	0.89 (0.38)	0.24 (0.11)	240.2 (114.2)	130.8 (54.3)
2.5	3.23 (0.64)	5.58 (1.35)	37.45 (25.91)	95.21 (64.27)	0.71 (0.28)	0.28 (0.12)	187.7 (57.6)	106.8 (25.0)
5.0	3.83 (0.21)	4.80 (0.92)	42.26 (5.44)	134.69 (9.61)	1.00 (0.12)	0.27 (0.08)	287.3 (56.2)	133.0 (29.8)
10.0	4.75 (1.16)	6.74 (3.22)	95.51 (42.28)	282.54 (100.67)	1.00 (0.40)	0.30 (0.16)	351.0 (126.0)	153.8 (51.4)
20.0	6.05 (3.08)	6.15 (2.32)	137.91 (8.97)	474.31 (232.52)	1.21 (0.09)	0.43 (0.21)	755.8 (623.6)	213.0 (60.5)
40.0	5.59 (1.74)	6.59 (1.65)	189.79 (80.10)	818.12 (359.98)	2.02 (1.05)	0.49 (0.27)	612.7 (92.7)	256.3 (18.4)

[1]Values are group (n=4-5) means and (SD).

As seen in Figure 4.10, a plot of enantiomeric AUC *vs.* enantiomeric dose (one-half racemic dose) is clearly not linear and therefore it is not surprising that a plot of clearance *vs.* enantiomer dose reveals that the clearance of the enantiomers is dependent on dose (Figure 4.11). Enhancement of clearance of the (R)-, and to a considerably less extent the (S)-enantiomer, upon increase in dose, is consistant of a stereoselective saturation of protein binding, as reported to be the underlying mechanism for non-linearity of naproxen (Runkel *et al.* 1973) and ibuprofen (Lockwood *et al.* 1983). However, unlike flurbiprofen in the rat, saturation of protein binding of ibuprofen in humans appears to be non-stereoselective, i.e. the two enantiomers deviate from non-linearity to the same extent (Jamali *et al.* 1992).

The observed dose-related progressive increase in clearance of (R)-FL also confirms the hypothesis that an interaction at the level of protein binding is the cause of the increased clearance of the (R)-isomer by co-administration of its antipode. Stereoselective protein binding for FL has been confirmed *in vitro*, with both enantiomers competing for a common binding site resulting in a mutual displacement from the plasma proteins of one enantiomer by the other (Knadler *et al.* 1988). The increased avidity of the (S)-isomer for plasma protein binding sites leads to stereoselective displacement of the enantiomers, becoming significant at concentrations greater than 26 mg/L (Knadler *et al.* 1988). This will result in a reduced number of plasma protein binding sites for the less avidly bound (R)-enantiomer with a preferential increase in its unbound fraction, leading to a relatively greater enhancement of its elimination and/or tissue uptake, than its antipode.

The observation of stereoselective dose-related clearance has several implications, the most important being that it is the apparently inactive (R)-enantiomer whose clearance is increased by co-administration of the (S)-isomer. As flurbiprofen fails to undergo inversion in man, it cannot be considered a pro-drug. Therefore, with increasing racemate dose (a common occurrence in arthritics with increasing disease progression), the AUC of the (R)-isomer increases in a non-linear manner (Figure 4.10) resulting in the plasma concentration of the (R)-enantiomer being lower than predicted if the AUC *vs.* racemic dose had been linear. However, the concentration of the active (S)-isomer will progressively rise in a linear manner. One can only presume that side effects may be less than expected (i.e., less than if the AUC *vs.* dose was linear)--assuming the (R)-isomer contributes significantly to overall toxicity. One could infer as the clearance of the (R)-isomer increases with increasing dose, the subsequent decrease in its steady-state plasma concentration may result in a less competitive interaction with its antipode with the possibility the concentration of (S)-isomer at the receptor level will increase. Therefore, as the racemic dose of FL increases, there may be a paradoxical decrease in anticipated toxicity with an accompanying increase in anticipated efficacy.

4.5 Presystemic and Systemic Chiral Inversion of R-(-)-Fenoprofen in the Rat

Following administration of racemic (10 mg/kg) and R-fenoprofen (R-FN) (5 mg/kg) through both IV and oral routes (Figure 4.12), the disappearance from plasma of the R-enantiomer occurred very rapidly and was generally undetectable 15 min after administration. It appears that the main elimination pathway of (R)-FN is through inversion to its antipode as its disappearance from plasma resulted in the subsequent appearance of substantial concentrations of (S)-FN (Figure 4.12). The declining phase of (R)-FN had a half-life of less than 5 minutes with no absorption phase detectable after oral administration. The fraction of dose inverted (fi) by the oral and IV routes was 0.46 and 0.42 respectively. The clearance and Vd of (S)-FN are listed in Table 4.7. In contrast to (R)-FN, the (S)-FN remained in plasma in substantial concentrations and was eliminated with a mean half-life of 10 hours (Table 4.7). Whether formed after (R)-FN or racemate IV administration, the clearance, t½, and V_{dss} of (S)-FN had the same magnitude (Table 4.7). However, the t½ of (S)-FN after (Rac)- and (R)-FN oral administration was significantly different which was due to a single rat showing a relatively high plasma concentration at the last sampling time. No significant difference was noted between the AUCs of (S)-FN after IV and oral administration of the racemate.

The observed rapid disappearance of (R)-FN and appearance of (S)-FN is also observed in man after oral administration of racemate (Rubin *et al.* 1985). The extent of inversion observed in humans (Rubin *et al.* 1985) was greater than that observed for the rat. This may be due to saturation of the inversion process after doses given in this study. This is supported by the observation with the isolated liver perfusion, that

Table 4.7 Pharmacokinetic parameters of (S)-fenoprofen following administration of (R)- and (Rac)-fenoprofen by the IV and oral routes						
Dose, mg/kg	Route	$t_{½}$, h	AUC, (mg/L)h	V_{dss}, L	Cl_{TB}, L/h	MRT, h
R, 5	oral	15.1 ± 4.2	211.0 ± 55.2	0.242 ± 0.131	0.011 ± 0.005	22.0
R, 5	IV	13.5 ± 7.2	167.5 ± 83.6	0.265 ± 0.211	0.013 ± 0.008	20.4
Rac, 10	oral	7.6 ± 2.6	665.0 ± 236.1	0.189 ± 0.104	0.012 ± 0.007	15.5
Rac, 10	IV	13.5 ± 3.8	561.7 ± 169.0	0.311 ± 0.095	0.015 ± 0.006	20.7

doubling the inlet concentration of (R)-FN did not lead to a corresponding increase of (S)-FN in the effluent (Figure 4.13b). Similar to the findings in this study with the rat, the half-life of (S)-FN in man is found to be substantially longer than that of (R)-FN (Rubin *et al.* 1985). The rat and human therefore display qualitatively similar fenoprofen plasma time-courses. Thus, the rat appears to be a suitable model for studying the disposition of unchanged FN enantiomers and their acyl glucuronides.

Assuming the liver as a major eliminating organ, a clearance of (R)-FN after po administration greater than the liver blood flow (1.74 mL/min/g liver, Daemen *et al.*, 1989) would indicate presystemic biotransformation. Such a possibility is usually delineated by comparing the AUC data after IV and oral administration as shown with ketoprofen (Foster and Jamali, 1988). In this case, however, the rapidity of the (R)-FN elimination makes the detection of the expected differences in AUCs after the two routes of administration difficult. Accordingly, no significant differences were found between the AUCs of (S)-FN after administration of racemate through IV and oral routes (Table 4.7); *i.e.* the extent of inversion is apparently the same regardless of the route of administration. Consequently, the contribution of individual organs to the inversion of fenoprofen was studied by the use of isolated perfused liver and everted intestinal tissue.

Direct evidence for the contribution of the liver to the inversion of the 2-APAs has been the subject of many studies, summarized in Table 1.2, which clearly show that inversion is dependent upon substrate, species and the method of tissue preparation. In the present study, single-pass (R)-FN perfusion of the isolated liver preparation clearly demonstrates significant R to S inversion (Figure 4.13). The single-pass infusion

of the (R)-FN was accompanied by a 1.5-2.0% impurity of the (S)-enantiomer (Figure 4.13B). Examination of single-pass data reveals that following perfusion of the liver with an initial inlet concentration of 7 mg/L of (R)-FN, there was a lag time of a few minutes before a measurable concentration of the (R)-enantiomer was found in the effluent (Figure 4.13A). As the steady-state concentration was only achieved after 15 minutes, this would indicate a considerable sequestering of the drug by the organ. Similarly, after discontinuing the infusion, the decline in effluent concentration was slow and measurable for several minutes thereafter. The decline of (R)-FN across the liver was accompanied by an increase of (S)-FN in the effluent, confirming the occurrence of enantiomeric inversion in the liver. With a one-fold decrease in the perfusion rate of (R)-FN, a corresponding decrease in the effluent concentration was observed, indicating linearity of clearance of (R)-FN at the concentrations studied. However, the decrease in perfusion rate of (R)-FN did not lead to a corresponding decrease in the effluent (S)-FN concentration, suggesting possible saturation of the inversion process at the higher rate. This was also observed when the liver was perfused from an initial low inlet concentration followed by a higher inlet concentration. The single-pass extraction ratio of (R)-FN ranged from 0.28 to 0.35, which corresponds to a hepatic clearance of 7.6 to 9.5 mL/min. The mean values of (R)-FN elimination t½ during and after the infusions were 3.8 and 3.4 min., respectively.

The lack of mass balance between the (R)-FN [plus (S)-FN impurity] inflow and the (S)- and (R)-FN outflow, coupled with the observed incomplete *in vivo* inversion is suggestive of involvement of pathways other than inversion for either with (R)-FN itself

or sequential metabolism of (S)-FN upon first pass. The latter seems unlikely as this enantiomer has a relatively low clearance in the intact animal (Table 4.6) and in the recirculation mode of the isolated perfused liver (Figure 4.14). A lack of mass balance between the inflow and outflow is evident; after correcting for the (S)-FN impurity, only 75% of the (R)-FN in the inflow is accounted for in the outflow as both (R)- and (S)-FN. A similar lack of mass balance between the loss of the (R)-isomer and rise of its antipode has been observed with ibuprofen using liver homogenate (Knihinicki *et al.* 1989; Knights and Addinall, 1990). In the present study, the observed lag time and the delay in achieving steady-state indicates a considerable uptake of the drug by the liver. However, the uptake and storage of drug by the liver is usually a reversible process and the irreversible removal of drug by the clearance processes are not affected by such uptake (Wedlund and Wilkinson, 1984). Therefore a second possibility for the lack of mass balance of the (R)-FN may be due its irreversible incorporation into cellular constituents, such as triacylglycerols as first reported with ibuprofen by Fears (1985 and references therein) and recently with fenoprofen in the isolated rat hepatocyte (Sallustio *et al.* 1988).

Stereoselective glucuronidation by the liver of a number of 2-APA members has been previously demonstrated or discussed (Bopp *et al.* 1979; El Mouelhi *et al.* 1987; Fournel-Gigleux *et al.* 1988). In the present study, substantial concentrations of conjugated (R)- and (S)-fenoprofen were formed after a single pass through the isolated liver (Figure 4.13A,B). This observation of first-pass glucuronidation with a 2-APA has not been reported previously. Comparison of effluent samples before and after NaOH

hydrolysis revealed that between 28 to 35% of the total (R)-FN recovered and 47 to 52% of the total (S)-FN recovered was present as the hydrolyzable conjugates. The large differences between the two enantiomers would indicate the first-pass conjugation is stereoselective with preference for the (S)-enantiomer. Interestingly, Fournel-Gigleux *et al.* (1988) using Wistar rat liver microsomes and 2-phenylpropionic acid as substrate, found a greater selectivity for the (R)-isomer.

In the effluent, (S)-FN was recovered as both the unchanged and conjugated-forms (Figure 4.13). However, it is not clear whether inversion preceded conjugation. The only possible location of ester glucuronide formation on the parent drug is via the carboxylic acid moiety attached to the chiral carbon. As the chiral carbon is the site of inversion, intuitively, the presence of the bulky glucuronide should prevent the approach of the enzyme(s) responsible for inversion. Using benoxaprofen as substrate, Bopp *et al.*, (1979) also suggested that inversion may occur prior to conjugation. In addition, since (R)-FN glucuronide was found in the effluent, this raises an interesting question: Does prior conjugation of (R)-FN prevent the inversion process? Examination of the recirculating liver perfusion (Figure 4.14) would indicate that it does not, as apparently all the circulating (*i.e.* non-sequestered) (R)-FN was eventually inverted to the (S)-antipode. There are therefore two possibilities: first, where a reversible futile cycle of conjugation-deconjugation (Schollhammer *et al.* 1975; Meffin *et al.* 1983) occurs during passage through the liver with inversion occurring when the drug is in the deconjugated state and/or secondly, the presence of the glucuronide does not impede the inversion process. The former process is more likely due to the unstable nature of acyl

glucuronides and presence of β-glucuronidase activity in the liver (Schollhammer *et al.* 1975; Meffin *et al.* 1983).

After a recirculating perfusion of rat liver with (R)-FN, a lag period is observed between the appearance of both enantiomers (Figure 4.14). Within 90 minutes after the start of the perfusion, (S)-FN concentration had reached a plateau whereas the presence of (R)-FN was not detectable. The mean t½ of (R)-FN elimination was 12.9 minutes with a mean 11.7 minutes t½ for the appearance of (S)-FN.

The calculated hepatic extraction ratio (0.28-0.35) is not sufficiently high to explain the rapid clearance of (R)-FN observed in the intact animal. This may indicate involvement of other organs such as the gastrointestinal tract as has been suggested for other 2-APAs (Jamali, 1988), for example, ketoprofen in rat (Foster and Jamali, 1988) and ibuprofen in man (Jamali *et al.* 1988; Borin *et al.* 1988).

The GIT has the ability to carry out a wide variety of Phase I metabolic reactions as demonstrated using various *in vitro* animal tissue preparations (Hartiala, 1973; Wollenberg and Ullrich, 1980). The enzymes capable of metabolizing xenobiotics in the intestinal tract may be of microbial and/or mucosal origin and present in the gut lumen and/or gut wall (Hartiala, 1973; Ilett *et al.* 1990). In addition, for many metabolic processes, the duodenum contains the highest enzymatic activities which progressively decrease along the length of the GIT (Ilett *et al.* 1990).

Upon administration of four different commercially available formulations of ibuprofen to humans, Jamali *et al.* (1988) found the longer the residence of drug in the intestinal tract, the greater the S:R concentration ratio becomes in plasma, suggesting

presystemic chiral inversion. Similar observations were reported by Borin *et al.* (1988) when the S:R AUC ratios of a sustained release formulation of ibuprofen were found to be significantly larger than that observed with a conventional dosage form. When ketoprofen was administered to Sprague-Dawley rats by the IV, intraperitoneal and oral routes, at least 84% of the administered dose of (R)-ketoprofen was shown to invert to the (S)-isomer *via* the GIT (Foster and Jamali, 1988). Simmonds *et al.* (1980) examined the inversion of benoxaprofen in both *in vivo* and various *in vitro* Wistar rat tissue preparations. This group showed that following a 4-h incubation of (R)-benoxaprofen with everted intestinal sac, 17% of total benoxaprofen was present as (S)-benoxaprofen.

The present study, using everted intestinal sacs, has demonstrated significant inversion of (R)-FN along the entire length of the intestine in all segments studied (Figure 4.15), on both the serosal and mucosal sides (Figure 4.16 and 4.17 respectively). Based on equivalent tissue weight, the jejunum displays the largest extent of inversion compared to the other segments (Figure 4.15), as evidenced by the largest S:R ratio on both the serosal and mucosal sides, with the duodenum exhibiting a relatively less ability to invert. Incubation of (R)-FN in stomach pouch (n=2, data not shown) failed to demonstrate (R)- to (S)-inversion. Mean ratios of total (S)-FN to total (R)-FN recovered on the serosal side were :duodenum, 1.2; jejunum, 2.2; ileum, 0.64; and colon, 0.23 with the ratios for the mucosal side: duodenum, 0.47; jejunum, 0.83; ileum, 0.33 and colon, 0.11 (Figures 4.16 and 4.17). This differs from the general findings regarding the distribution of metabolic activities discussed above. Interestingly, the S:R ratio for the serosal side is significantly greater than that found on the mucosal side (Figures 4.16

and 4.17). This may indicate inversion occurs as (R)-FN diffuses from the mucosal to serosal side. Therefore the site of inversion apparently may not be on the apical surface of the epithelial cell (*i.e.* brush border) but either inside the enterocyte (cytosol or organelles) or the baso-lateral membrane. However, glucuronide conjugates may also be actively transported to both the serosal and mucosal sides (Koster 1985; Koster and Noordhoek, 1983b).

Extent of inversion must depend greatly on the time the drug is in contact with the absorbing surface. Therefore, the use of isolated everted gut preparations where all tissue segments were incubated to the same extent may not mimic that seen *in vivo*, where drug contact with the various segments of the GIT is quite variable. Therefore, the results concerning the relative amount of inversion seen in each tissue segment may not reflect the *in vivo* situation and must be viewed with caution.

Another suggested explanation which has received little attention is the possibility of post-absorptive gastrointestinal inversion, that is, inversion by the GIT *via* extra-biliary excretion of the 2-APAs in the direction of blood to intestinal lumen. Such a pathway of metabolism has been observed for the cardiac glycosides (Lauterbach, 1981) and sulfonic acids (Sund and Lauterbach, 1978). Preliminary work in this laboratory has suggested this possibility.

In addition to the Phase I metabolic reactions in the intestine, Phase II activities such as glucuronidation can be found along the intestine of several species (Koster 1985). β-glucuronidase activity in the small intestine is reported as increasing from the oral to the distal end (Hanninen *et al.* 1968). Also, variable UDPGT activity can be found from

the mouth to the colon which parallels the observed decrease in glucuronidation capacity of a number of drugs from the duodenum to terminal ileum (Dawson and Bridges, 1979; Koster *et al.* 1985; Hanninen *et al.* 1968; Aitio and Marniemi, 1980). However, these findings may be dependent on the substrate and tissue preparation studied (Koster and Noordhoek, 1983; Lasker and Rickert, 1978). In the present study, glucuronidation was observed in all GIT tissues examined, although with considerable variability (Figure 4.18). The conjugate:unchanged ratio for total drug was highest for the duodenum and progressively decreasing along the intestine toward the colon, similar to the general finding discussed above.

A comparison of the serosal conjugate:unchanged ratio (Table 4.8) of the (S)-enantiomer with its antipode revealed significant preferential glucuronidation for the (S)-enantiomer. Interestingly, although glucuronidation was observed in the colon (Figures 4.16 and 4.17) the glucuronidation is apparently non-stereoselective as evidenced by the similar conjugate:unchanged ratio (Table 4.8), in contrast to that observed with the three other intestinal segments. The relative amount of glucuronidation was therefore dependent on the intestinal section. A tissue which exhibits stereoselective glucuronidation will display significantly different conjugate:unchanged ratios for each enantiomer. The ratios of each enantiomer for a non-stereoselective process will be identical as the fraction of each enantiomer undergoing glucuronidation will be similar. Similar findings were observed on the mucosal side. Total enantiomer (sum of serosal and mucosal sides, Figures 4.16 and 4.17 respectively) is also shown in Table 4.8.

A comparison of total (sum of S and R enantiomers) quantity recovered as

Table 4.8 Fenoprofen Enantiomer Conjugate:Unchanged Ratios of Everted Gut Mucosal and Serosal Solutions						
	Serosal		Mucosal		Total	
Tissue	S	R	S	R	S	R
Duodenum	1.44	0.42	1.01	0.35	1.04	0.35
Jejunum	1.45	0.88	0.71	0.15	0.78	0.18
Ileum	0.30	0.23	0.88	0.08	0.82	0.08
Colon	0.50	0.65	0.12	0.16	0.16	0.18
* Expressed as total enantiomer (sum of serosal and mucosal sides, Figures 4.16 and 4.17)						

conjugates:unchanged fenoprofen ratios (from figure 4.18) for the intestinal tissues(duodenum, 0.52; jejunum, 0.70; ileum, 0.23 and colon, 0.19) was highest for the jejunum, followed by the duodenum, ileum and colon.

In the present study, the Sprague-Dawley rat serves as a good model for the study of inversion of (R)- to (S)-FN in man. However, as inversion is dependent on animal species and substrate, these results should not be extrapolated to other 2-arylpropionic acids or species.

Through the use of isolated liver and everted intestinal sacs, it is clear that both organs are capable of substantial pre-systemic (R)- to (S)- inversion. In addition, certainly the liver and possibly intestine invert (R)-FN during systemic circulation.

V. Summary

The purpose of this research was to investigate the disposition kinetics and the inversion process of selected 2-arylpropionic acids: flurbiprofen and fenoprofen, in man and/or a suitable animal model. The specific systematic approach detailed in this investigation, involved a sequence of steps:

1. The following information was obtained from the literature.

a) A large percentage of the clinically administered pharmaceuticals are chiral and administered as racemates, but as a group relatively little is known about their enantioselective pharmaco-kinetics and -dynamics. Perhaps the principal reason for the void was the lack of suitable analytical methodology, which only became readily available over the last decade. Increased capabilities in analytical liquid chromatographic technology has given virtually any laboratory the capability to carry out enantioselective studies. This has led to an immense outgrowth in pharmacokinetic/dynamic investigations causing enantioselective studies to become one of the most favoured areas of research. As expected, much of the research being conducted during this development period is empirical data collection in small group experimental situations.

b) The 2-APAs have become the subject of intense study as each enantiomer of a racemate has the potential for exhibiting quite different pharmacokinetics from the other. Of the 2-APA's studied to date, no two agents have displayed similar stereoselective pharmacokinetic patterns. This is believed due, in part, to the presence of the chiral centre. All 2-APA members have the *in vivo* potential to undergo inversion from the (R)-enantiomer to its antipode (or also the (S)- to the (R)-enantiomer) by the chemical

nature of the propionate chiral carbon and the enzyme responsible for inversion. However, the provision to undergo inversion is lacking in several of the 2-APAs. It is clear that the extent of inversion is dependent upon drug, species and when examined *in vitro*, by tissue preparation. In addition, enantio-selective protein binding, glucuronidation and metabolism has been observed with most of the 2-APAs studied and therefore any or all of these processes may contribute to the stereoselective pharmacokinetics observed by this class.

c) It was clear that it was not possible to make class-wide generalizations, either with respect to their individual disposition pharmacokinetics or to the relative extent of inversion. Consequently, individual studies must be carried out with each member of this class in order to adequately ascertain their respective pharmacokinetics.

2. For the present study, a member of the 2-arylpropionic class whose enantiomeric pharmacokinetics had not been previously examined in any species was chosen.

a) Flurbiprofen had recently been given market approval by the Canadian Health Protection Branch and at the same time was undergoing phase III studies in the U.S. Therefore, the approximately simultaneous release onto the North American market made an enantiomeric investigation of flurbiprofen timely.

b) Using a developed stereospecific HPLC assay, plasma and urine concentrations of FL enantiomers were quantified to study the stereoselective pharmacokinetics in humans and the Sprague-Dawley rat. Both man and rat exhibited stereoselective disposition kinetics, with the (R)-enantiomer exhibiting a larger clearance than its

antipode. Furthermore, there was non-detectable and minimal inversion, in man and the rat respectively.

As there is minimal inversion of flurbiprofen, an alternate agent was needed to study the enantiomeric inversion process, in particular, the location of inversion. To this end, fenoprofen was the most plausible candidate, as earlier work by investigators clearly demonstrated that this agent underwent extensive inversion in man. Using the Sprague-Dawley rat as a model, after *in vivo* oral and intravenous administration, fenoprofen was found to undergo extensive (R)- to (S)- inversion by both routes. Individual isolated Sprague-Dawley everted gut segments (duodenum, jejunum, ileum and colon) exposed to (R)-FN all displayed the ability to carry out extensive inversion and glucuronidation. The latter conjugation reaction was shown to be stereoselective in the three small intestinal segments but non-stereoselective in the colon. Both the single-pass and the recirculating perfused liver, displayed considerable inversion and first-pass glucuronidation. These findings are indicative of both a presystemic and systemic involvement in the inversion process.

c) An observation in man after administration of racemate and single (R)-enantiomer led to a further study in the rat which demonstrated an *in vivo* interaction between FL enantiomers. This resulted in a mutual alteration of disposition kinetics after racemate administration: the mean AUC of the (R)-isomer decreased upon co-administration of the (S)-isomer. In addition, a trend for the decrease in the AUC of the (S)-isomer with co-administration of the (R)-isomer was also observed.

d) With the possibility the enantiomeric interaction may be dose-related, six

different doses of racemate FL were administered intravenously to the rat. The enantiomeric disposition kinetics of the enantiomers revealed the clearance of the (R)-enantiomer was increased with escalating racemic dose. Interestingly, the (S)-enantiomer failed to exhibit a similar significant change in clearance. The stereoselective enhancement of (R)-FL clearance is suggested to be due to saturation of protein binding sites which may further confirm the hypothesis that the enantiomeric interaction observed previously was at the level of protein binding.

3. Inference from the above findings include:

a) The largest disparity between the *in vivo* and *in vitro* eudismic ratios (8.1 and 878 respectively is observed with flurbiprofen. However, the minimal inversion shown by the present work questions the explanation of inversion being the cause of the eudismic ratio discrepancy, at least in the case of flurbiprofen. This example contributes to the hypothesis that the neutrophil is a major *in vivo* inflammatory mediator as opposed to PGs *in vitro*. The many *in vivo* eudismic ratios close to unity offer evidence that there is minimal stereoselectivity with regard to the stimulus-response uncoupling. In view of the increasing evidence regarding the importance of the neutrophil-NSAID interaction in inflammation, a reappraisal of *in vitro* assay methods for the examination of anti-inflammatory activity is clearly warranted.

b) Our observations present yet another example of the complex pharmacokinetics of the 2-APA and other chiral drugs that are administered as racemates. Interestingly, the reported enantiomeric pharmacokinetic patterns of not even two of these chemically

related chiral NSAIDs are similar, therefore it is not possible to make class-wide generalizations. Also, the metabolic bio-inversion of one enantiomer to the other observed for some of these compounds cannot be extrapolated to others as evidenced by the extensive inversion of fenoprofen and the essentially complete deficiency for flurbiprofen.

Consequently, it is clear that individual studies must be carried out with each member of this class in order to adequately ascertain their respective pharmacokinetics. In addition, stereospecific assays are appropriate for each individual member of this class, with the possible exception of tiaprofenic acid. Indeed, the differential pharmacologic activity of the (R)- and (S)- enantiomers makes such studies even more relevant, not only among NSAIDs, but also among other therapeutic agents administered as racemate.

c) As discussed in the section 1.4, there is generally an absence of a relationship between therapeutic response and plasma concentrations of the 2-APAs. When discussing the pharmacokinetics of the racemate *vs.* the single enantiomers, one must ensure the disposition of the same isomers are equivalent. Evidence of an *in vivo* enantiomeric interaction at the level of any pharmacokinetic process is indicative that a similar interaction may also be occurring at the pharmacodynamic level, that is, at the receptor. This effects a major assumption fundamental to pharmacokinetics--the concentration at the receptor is in equilibrium or associated with the concentration at the sampling site. An enantiomeric interaction may negate the ability to deduce a dose-response relationship, thereby limiting the ability of pharmacokinetics to make inferences

with respect to therapeutic dose regimens.

d) Stereoselective dose-related clearance has a special significance. With increasing disease progression, the dose of NSAID is also increased proportionally. If the findings in the Sprague-Dawley rat can be extrapolated to man, one possible outcome of progressive dose increases may be a lower-than-expected toxicity caused by the lower-than-expected steady-state concentration of the non-inverting inactive isomer, and perhaps a higher-than-expected increase in efficacy of the active (S)-isomer incited by the decreasing competitive interaction between the two isomers.

e) The observation of presystemic inversion with fenoprofen, if extrapolatable to man and other 2-APAs, can have two major implications. First, it may have influence on dosage form design for those agents that have demonstrable *in vivo* inversion, as pre-systemic inversion increases, so does the concentration of active isomer. Second, the effect of the individual enantiomers on the local GIT toxicity has not been clearly established. In addition, the effect of age on inversion has not been examined. In view of the lack of knowledge in these areas, any pre-systemic inversion may have important implications with regard to toxicity and/or efficacy.

f) The results of the fenoprofen studies demonstrate that the Sprague-Dawley rat serves as a good model for study of i) the *in vivo* pharmacokinetics and ii) the inversion process because of the general similarity of the processes in man.

g) The results of the fenoprofen work may have offered some insight into the phenomena of hybrid triglyceride formation. Recall (cf. section 1.4.3.1) that the acyl-CoA thioester formation has been shown by several workers to be an obligatory

intermediate during the (R) to (S) inversion process. Consequently, the extent of the uptake by the adipose and hepatic tissues may be dependent upon the degree of the enantiomeric inversion. Interestingly, ketoprofen which has been shown to undergo 80% (R) to (S) inversion in the rat is taken up into adipose tissue to a considerably greater extent than is flurbiprofen which undergoes only a limited extent of inversion. For ketoprofen the occurrence of inversion means (R)-isomer thioester formation preceded inversion and a proportion of the formed (R)-thioester was subsequently incorporated into hepatic tissue as the hybrid triglyceride. For fenoprofen, whose extent of inversion in the rat approaches 100% and therefore exceeds the extent of ketoprofen inversion, also exceeded ketoprofen in the degree of hepatic tissue uptake therefore indicating a direct correlation. These findings also demonstrate that although a drug may exhibit a rapid and extensive inversion to the non-hybrid forming (S)-enantiomer, there is considerable tissue incorporation. It has been suggested that upon administration of an (R)-enantiomer, it may undergo an initial sequestration into fat which acts as a depot initially protecting it against inversion. However, evidence against this hypothesis is two-fold: the observed rapid rate of inversion of fenoprofen and the apparent inability of some xenobiotic hybrid triacylglycerols to be substrates for the enzymatic degradation by lipases, therefore precluding an initial rapid hybrid uptake, release and finally inversion.

h) A more plausible explanation for the relative differences in 2-APA uptake as hybrid triglycerides and its correlation with the extent of inversion may involve saturation of one of the inversion stages: either the acyl-CoA synthetase, the "epimerase" or the acyl-CoA hydrolase step. The enzyme-dependent epimerization step (*i.e.* R-CoA ———»

S-CoA) is most likely the rate-determining step. If the synthetase were rate-limiting the formed CoA thioesters would probably be immediately inverted to the (S)-isomer allowing little to be taken up as hybrid triglycerides. Conceivably, a particularly good substrate for the acyl-CoA synthetase such as fenoprofen, may result in such a rapid increase in the (R)-FN-CoA concentration that it saturates the epimerase, causing a build-up of the (R)-FN-CoA. The formed excess may now become a substrate for incorporation into the hybrid triglycerides. Furthermore, the inability of the (R)-CoA to act as a substrate for amino acid conjugation (c.f. section 1.4.2.3) would further promote the supposed shunting of the excess (R)-CoA into the hybrid triglyceride pathway. Therefore, the better the substrate for acyl-CoA synthetase (*i.e.* the faster the rate of formation of the 2-APA-CoA thioester), the greater potential for epimerase saturation, and subsequently the more (R)-CoA would be available for shunting into the hybrid triglycerides. Evidence for the presence of a saturation process is seen after fenoprofen infusion into an isolated rat liver. Here, a doubling of the fenoprofen infusion rate failed to cause a matching increase in the appearance of (S)-FN in the effluent. Further research is needed in this area.

VI. References

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